Synthetic Studies of Biologically Active Natural Products – Ascididemin and 6-Substituted 2-Pyranones

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Abstract

Synthetic studies of two classes of natural products, ascididemin and 6-stryl-4-methoxy-2-pyranone, are described. Compounds produced in these studies were submitted to a number of biological assays.

The marine alkaloid ascididemin has considerable activity against *Mycobacterium tuberculosis* but unfortunately possesses significant cytotoxity and poor solubility. Analogues of ascididemin were prepared with variations at the 6-position and, in many instances, with replacement of the nitrogen at the 8-position with a carbon. Substituents were introduced that explored steric bulk and also enhanced aqueous solubility. Two new general routes were developed for the preparation of amide analogues at the 6-position of ascididemin. A set of 6-amidostyryl-8-deaza-ascididemin analogues showed considerable activity (MIC MTb 0.2-0.7 μM), good solubility and modest to good selectivity (SIs from 6 to 125).

2-Pyranone natural products **1.14** and **1.15** have been reported to exhibit modest in vitro activity against *Mycobacterium tuberculosis* and the related 2-pyranones pseudopyronines A (**1.27**) and B (**1.28**) and analogues are reported to have anti-parasitic activity. To explore the structure activity relationship of these compounds a library of 6-substituted-4-methoxy-2-pyranones was prepared. Significant in vitro activity against *Plasmodium falciparum* was observed for several members of the compound library (*e.g.* **3.55** and **3.61** with IC_{50} values of of 1.3 and 3.6 μM, respectively). Biomimetic photo-dimerisation of several styryl pyrones was explored and isolated dimers submitted to assays. X-ray crystal structures of two of the monomers were used to rationalise the resulting dimer structures. These dimerised pyrones were found to be generally more active against *P. falciparum* and less toxic than their monomers and had the best selectivity of the pyrone library evaluated.

Declaration and preface

This is to certify that:

1) the thesis comprises only my original work towards the PhD except where indicated below;

2) due acknowledgment has been made in the text to all other material used.

Biological testing of compounds was performed by various collaborators:

Dr. Helena Boshoff and Dr. Clifton E. Barry III at the National Institute of Allergy and Infectious Diseases, NIH, USA tested compounds in *Mycobacterium tuberculosis* H37Rv assays.

Dr Ronan O'Toole, School of Biological Sciences, Victoria University of Wellington tested compounds in *Escherichia coli*, *Mycobacterium smegmatis* and HL-60 assays;

Ms Gill Ellis, Department of Chemistry, The University of Canterbury evaluated P388 activities of compounds.

Drs Marcel Kaiser and Reto Brun, Department of Parasite Chemotherapy of the Swiss Tropical and Public Health Institute, Basel, Switzerland evaluated compounds against *Trypanosoma brucei rhodesiense, Leishmania donovani*, *Plasmodium falciparum* and an L6 rat skeletal myoblast cell line.

Acknowledgements

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I would also like acknowledge various collaborators for the biological evaluation of various compounds: Dr. Helena Boshoff and Dr. Clifton E. Barry III at the National Institute of Allergy and Infectious Diseases, NIH, USA for the testing of compounds in their *Mycobacterium tuberculosis* H37Rv assays; Dr Ronan O'Toole, School of Biological Sciences, Victoria University of Wellington for the testing of compounds in *Escherichia coli*, *Mycobacterium smegmatis* and HL-60 assays; Ms Gill Ellis, Department of Chemistry, The University of Canterbury for the evaluation of P388 activities; Drs Marcel Kaiser and Reto Brun, Department of Parasite Chemotherapy of the Swiss Tropical and Public Health Institute, Basel, Switzerland for the evaluation of compounds against *Trypanosoma brucei rhodesiense, Leishmania donovani, Plasmodium falciparum* and an L6 rat skeletal myoblast cell line.

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Finally I would like to acknowledgement my family, whose love and understanding have kept me going throughout my work.

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Abbreviations

Ac	acetyl
ACT	artemisinin-based combination therapy
ATCC	American Type Culture Collection
ATR	attenuated total reflectance
br	broad
BCG	bacillus Calmette-Guérin
BSA	bovine serum albumin
C_8	octyl-derivatized silica
C_{18}	octadecyl-derivatized silica
CHO	Chinese hamster ovary
CI	chemical ionization
COSY	gradient correlation spectroscopy (^{1}H-^{1}H)
d	doublet
dn	n deuterium atoms present in the molecule
DCC	*N*,*N'*-dicyclohexylcarbodiimide
DCU	*N*,*N'*-dicyclohexylurea
DNA	deoxyribonucleic acid
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMF-DEA	dimethylformamide diethyl acetal
DEPT	distortionless enhancement by polarisation transfer
DMSO	dimethylsulfoxide
DOTS	directly observed therapy short course
EC_{50}	half maximal effective concentration
ED_{50}	50 % effective dose concentration
EI	electron impact
ELISA	enzyme-linked immunosorbent assay
ESI	electrospray ionization
Et	ethyl
et al.	et alia
FAB	fast atom bombardment

g	gram
h	hours
H_{37}Ra	an avirulent strain of *Mycobacterium tuberculosis*
H_{37}Rv	a well characterised virulent strain of *Mycobacterium tuberculosis*
HIV	human immunodeficiency virus
HMBC	gradient heteronuclear multiple-bond correlation
HPLC	high performance liquid chromatography
HR	high resolution
HRP2	histidine-rich protein II
HSQC	gradient heteronuclear single-quantum correlation
Hz	hertz
IC_{50}	50% inhibitory concentration
IR	infrared
J	coupling constant
J_{AB}	coupling constant between atoms A and B
L	litre
LH20	Sephadex LH20
M	molarity
m	metre
m	milli
m	multiplet (NMR)
m	meta
MDR	multidrug-resistant
Me	methyl
MeCN	acetonitrile
MeOH	methanol
MIC	minimum inhibitory concentration
min	minutes
m.p.	melting point
MS	mass spectrometry
MTb	*Mycobacterium tuberculosis*
m/z	mass to charge ratio
n	nano
N	normal

NBS	N-bromosuccinimide
NCI	National Cancer Institute
NMR	nuclear magnetic resonance
NOE	Nuclear Overhauser Effect
NOESY	Nuclear Overhauser Effect Spectroscopy
o	ortho
p	para
ppm	parts per million
R_f	retention factor
RNA	ribonucleic acid
ROESY	rotating-frame nuclear Overhauser enhancement spectroscopy
r.t.	room temperature
s	singlet
SAR	structure-activity relationship
SI	Selectivity Index
sp.	species
t	triplet
tert	tertiary
TB	tuberculosis
TBDPS	*tert*-butyldiphenylsilyl
Tf_2O	trifluoromethanesulfonic anhydride
TFA	trifluroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
UV	ultraviolet
W	watt
WHO	World Health Organization
XDR	extensively drug-resistant
Å	angstrom
2D	two dimensional
^{1}H NMR	proton nuclear magnetic resonance
^{13}C NMR	carbon-13 nuclear magnetic resonance
δ_A	NMR chemical shift in ppm downfield from a standard for nucleus A

μ	micro
Δ^n	chemical bond from position n to n + 1
ν_{max}	Wavenumber with local maximal intensity

Chapter 1 Introduction

1.1 Third world disease

Several contagious and severely pathogenic diseases threaten the health and prosperity of the world's poorer nations. Millions die each year, primarily in developing countries from these diseases which include tuberculosis, malaria, trypanosomiasis (Chagas Disease and Sleeping Sickness) and leishmaniasis. Tuberculosis alone kills more than 1 million a year and the death rate from malaria is just under that figure.[1,2] In addition several neglected diseases ravage these nations including leishmaniasis and trypanosomiasis.[3-5] While the World Health Organisation (WHO) monitors and treats, where possible, outbreaks of these diseases, new pharmaceuticals that can more effectively and more economically treat these conditions are required. The rise of resistance to existing therapies to some diseases, especially tuberculosis and malaria, provides a constant need for new potent drugs to treat these conditions. The development of new therapies for these diseases offers relatively little return on investment and as such is primarily left to academic, government and charitable organisations.

1.2 Tuberculosis

Tuberculosis is a contagious and potentially lethal disease caused by the Gram-positive bacterium *Mycobacterium tuberculosis*. The bacterium is spread from host to host via droplets expelled from an infectious individual, during coughing or sneezing. Once an individual is exposed to *M. tuberculosis*, the disease lies dormant in the infected person's lungs. Approximately 5-10% of infected people go on to develop active tuberculosis in their lifetime and in this active state the disease becomes highly contagious and life-threatening. Overall, one third of the global population is currently infected with tuberculosis and WHO estimates that 1.3 million people died from the disease in 2008.[1]

Latent tuberculosis can be activated when the immune system is compromised, and consequentially, HIV sufferers are 20 to 40 times more likely to develop active tuberculosis.[6] Treatment of tuberculosis in communities with significant HIV

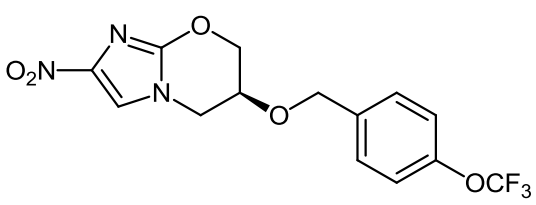
infection rates requires more aggressive and tightly managed approaches. Some of the current drugs used to treat tuberculosis are incompatible with common anti-retro viral treatments used to control HIV and consequently longer, more expensive and less pleasant regimes are often required for HIV-infected individuals. Accordingly new drugs compatible with anti-retro viral regimes are needed to treat tuberculosis in HIV patients.[1]

The rise of drug resistant strains, particularly multi-drug resistant strains (MDR) where the strain is resistant to two or more front line drugs, of tuberculosis requires treatment regimes using second-line drugs which are less potent and have more side-effects than the front-line therapies. While MDR tuberculosis strains remain treatable, poor drug management and incomplete treatment of these strains has lead to the emergence of extensively drug-resistant (XDR) tuberculosis. These strains of tuberculosis have resistance to second line drugs on top of the front line drugs and are very difficult to treat, if treatable at all. Controlling the spread of these organisms is difficult with the limited useable pharmaceuticals and the consequential long and costly treatment regime. New drugs with novel mechanisms of action are needed to meet this growing threat.[1,6,7]

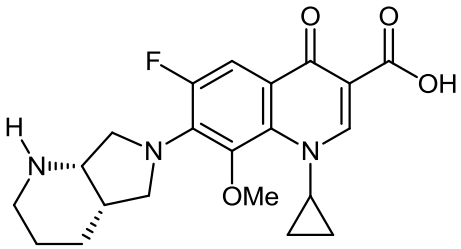
1.2.1 Current therapies and new developments

The current therapy of tuberculosis involves a 6 month course of four front line drugs, isoniazid, ethambutol, pyrazinamide and rifampin, following the WHO's directly observed therapy – short course (DOTS) protocols. The tuberculosis being treated is evaluated for resistance to these drugs and if found, the drug is replaced by one or more of second line drugs, which have less activity and/or greater side-effects. Natural products and drugs developed from natural products form the core of these therapeutics. Natural products and/or their derivatives in current use include rifampicin, streptomycin, amikacin, viomycin, capreomycins, kanamycin and cycloserine, which exhibit MICs of 0.2 $\mu g\ mL^{-1}$ (rifampicin), 0.5 $\mu g\ mL^{-1}$ (streptomycin), 1 $\mu g\ mL^{-1}$ (amikacin), 4 $\mu g\ mL^{-1}$ (viomycin), 5 $\mu g\ mL^{-1}$ (capreomycins), 6 $\mu g\ mL^{-1}$ (kanamycin) and 10 $\mu g\ mL^{-1}$ (cycloserine).

Several compounds are under development for tuberculosis treatment. The nitroimidazole PA 824 (**1.1**) and the 8-methoxy fluoroquinolone moxifloxacin (**1.2**) are two examples currently in late clinical trials, and are examples of developments being undertaken by the TB Alliance.[8-11]



1.1



1.2

PA 824 is the first compound in the TB Alliance portfolio. The nitroimidazole exhibits activity against all known resistant strains of *Mycobacterium tuberculosis* with high selectivity. It entered clinical trials in 2005 and phase II clinical trials are ongoing.[9,10] Moxifloxacin is currently in phase III clinical trials and is expected to become part of common treatment regimes in the near future.[8,11]

1.2.2 Anti-tuberculosis drug discovery

Discovery of new drugs requires an interdisciplinary approach where chemists develop or isolate drug candidates that are in turn evaluated by biologists for their activity against the pathogen along with selectivity and, if the provisional results are encouraging, the bioavailability and efficacy *in vivo*. This process has two distinct parts. The first is the identification of novel/lead compounds, and the second is the optimisation of these leads via analogue synthesis.

Natural products are an ongoing source of potential drug leads. Many lead-compounds are found by the broad analysis of an isolated and fully characterised natural product against a host of targets, but the drive to find compounds active against a specific target has prompted the use of bioassay-directed fractionation. This iterative process starts by taking extracts from natural sources and evaluating them for bioactivity against a selected target (*e.g. M. tuberculosis*). Active extracts are then

fractionated and the fractions evaluated for activity. Active fractions are further fractionated and this process is repeated until the compound(s) responsible for the activity is isolated and, in turn, elucidated. This methodology has become viable through advancements in high-throughput screening as well as advancements in the fields of chromatography (especially HPLC) and spectroscopy, most notably in MS and NMR techniques.

Ultimately, any compound developed for treating tuberculosis must have activity against *Mycobacterium tuberculosis* in the host. In order to reflect this, assays used in initial screening utilise either a virulent strain of *M. tuberculosis* or a closely related organism. A well characterised virulent strain is H_{37}Rv (ATCC 27294), however handling this organism requires a biosafety level 3 laboratory not commonly available at research institutes. Collaboration with groups that have access to this organism is one solution to this problem. Many groups, however, work with avirulent surrogate organisms. Commonly used surrogate species include *Mycobacterium smegmatis* (ATCC 607), the avirulent strain *M. tuberculosis* H_{37}Ra (ATCC 25177) and the commonly used vaccine strain *Mycobacterium bovis* BCG (ATCC 35743).[12]

Several bioassays have become popular in antimicrobial analysis and these vary in the information they provide as well as in the cost and time taken to aquire results. Agar diffusion is a relatively simple and robust methodology where zones of growth inhibition are measured from the site of chemical dose. This method works well in indicating the presence or absence of activity and as an early indicator for further analysis, and is most useful when evaluating drug resistance of clinical strains to commonly used antibiotics. Quantitative data is, however, difficult to acquire through this method, especially since the zone of inhibition is dependant on the compounds ability to diffuse through the agar plate. An advance on this methodology is the use of macro and micro agar dilution where the compound is mixed into the growth medium at a known concentration. This allows for quantitative activity to be determined though a main drawback is the 18 days needed for visible colony growth of *Mycobacterium tuberculosis*. Micro broth dilution allows for analysis of small sample sizes, is low cost and can be performed in high through-put. Growth measurements are readily evaluated with the aid of dyes that can be measured visually or by absorbance spectroscopy as well as by fluorescence spectroscopy.[12]

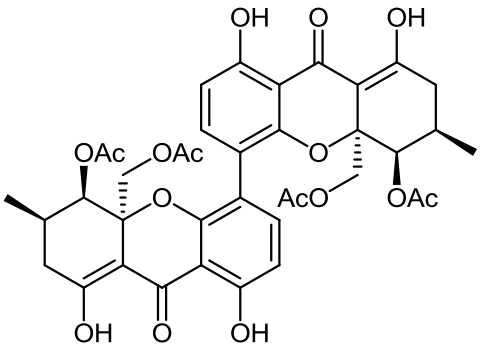
Alternatively, genetically modified mycobacteria, containing fluorescent or luminescent protein genes, can be counted and located by photometric detection.[13] These reporter gene assays offer a means to determine growth inhibition in complex environments such as *M. tuberculosis*-infected macrophages.[14] Assays exploring low-oxygen anti-*M. tuberculosis* activity have been utilized to evaluate compounds for treatment of the dormant form of *M. tuberculosis* that is present in individuals with latent tuberculosis.[12,15]

All of these assays enable the evaluation of compounds for anti-*Mycobacterium tuberculosis* activity and when combined with a measure of cytotoxicity against mammalian cells provide an outline of the potential of a compound *in vitro*.[12] Promising compounds are in turn taken to *in vivo* animal models, and finally enter in clinical trials. Clinical trials for *M. tuberculosis* have an added complication in that the disease is treated by a multi-drug regime and new drugs are trialed as substitutions for one member of this drug regime. This can mean many drug trials are undertaken before the compound is approved for front-line use.[16]

1.2.3 Natural product lead compounds

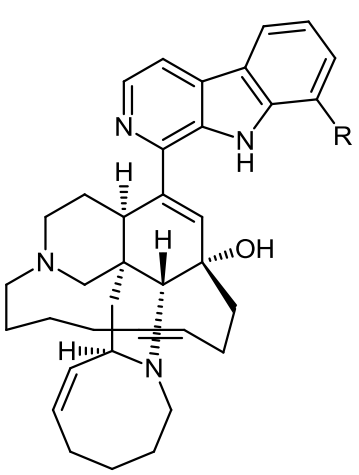
Natural products have long been a source of novel antibiotic compounds. Numerous examples of natural products have been reported to have significant anti-*Mycobacterium tuberculosis* activity and form the basis for many ongoing studies in the search of new anti-tubercular drug therapies.[17,18] As illustrated by the following examples, natural products offer a diversity of chemical structures with, in some cases, potent biological activities.

Phomoxanthone A (**1.3**) is a xanthone dimer isolated from the fermentation of an endophytic fungus of the genus *Phomopsis* and exhited potent activity against *Mycobacterium tuberculosis* H_{37}Ra with an MIC of 0.5 μg mL^{-1}.[19]

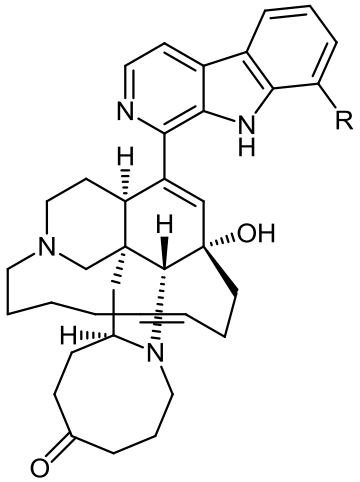


1.3

The manzamine family of marine akaloids have been isolated from a variety of marine sponges and are reported to have antimycobacterial activity. Examples shown (**1.4**-**1.7**) have MICs of 1.53 (**1.4**), 0.91 (**1.5**), 3.76 (**1.6**) and 2.56 (**1.7**) μg mL^{-1} towards *Mycobacterium tuberculosis* $H_{37}Rv$.[20-24]

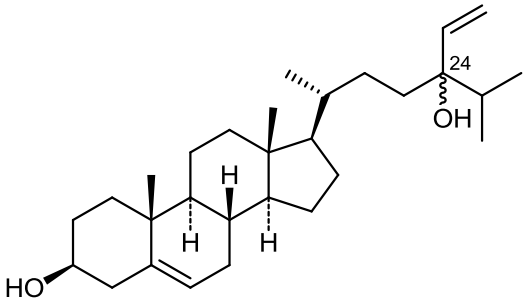


1.4 R = H
1.5 R = OH



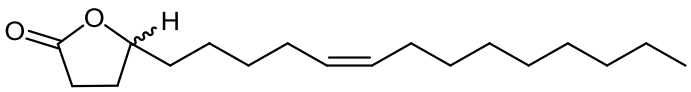
1.6 R = H
1.7 R = OH

Saringosterol is a 1:1 mixture of the C-24 epimers (**1.8**) 24*R* and (**1.9**) 24*S* first isolated from the brown alga *Sargassum ringoldianum*.[25] The mixture of metabolites exhibited an MIC against *Mycobacterium tuberculosis* $H_{37}Rv$ of 0.25 μg mL^{-1} with low toxicity towards the Vero cell line (IC_{50} > 128 μg mL^{-1}). Further studies determined the 24*R* isomer to be the more active component (MIC 0.125 μg mL^{-1}) with the 24*S* isomer eight times less active (MIC 1 μg mL^{-1}).[26] The high selectivity (SI > 500) of this compound shows significant potential for development.



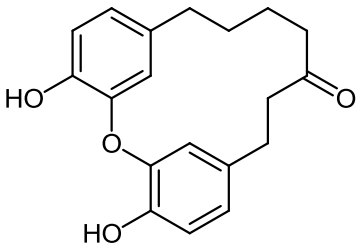
1.8 24 *R*
1.9 24 *S*

Micromolide (**1.10**), isolated for the stem bark of *Micromelum hirsutum*, exhibits an MIC of 1.5 μg mL^{-1} against *Mycobacterium tuberculosis* $H_{37}Rv$ with mild cytotoxicity towards the Vero cell line (IC_{50} 95 μg mL^{-1}) and has therefore a degree of selectivity towards *M. tuberculosis*.[27]



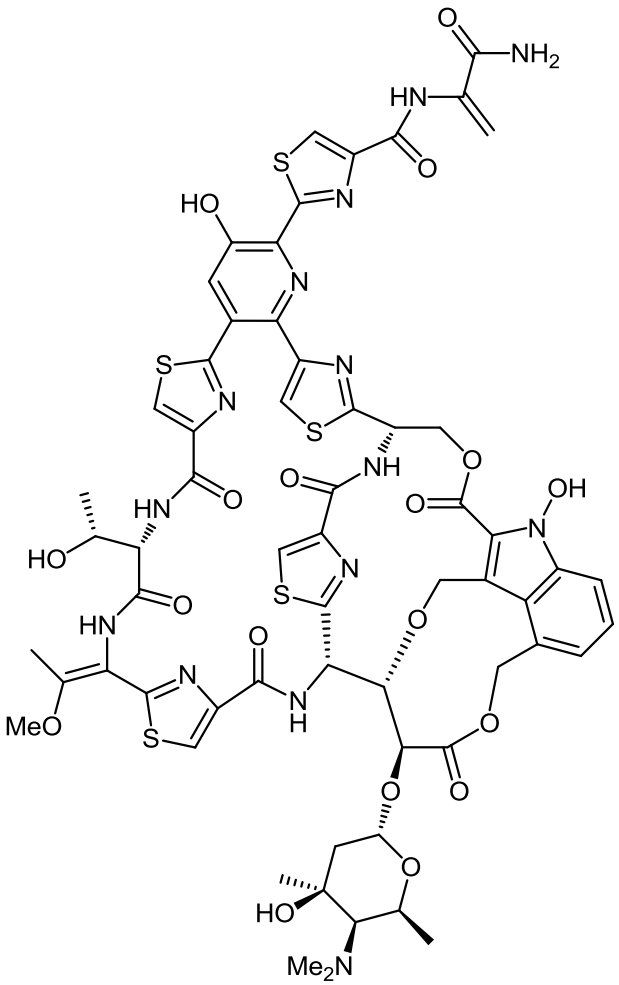
1.10

Like micromolide, engelhardione (**1.11**) represents a relatively simple lead compound with potent activity against *Mycobacterium tuberculosis* $H_{37}Rv$ with an MIC of 0.2 μg mL^{-1}.[28]



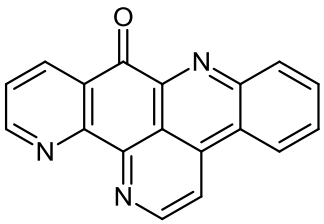
1.11

The structurally complex peptide nocathiacin I (**1.12**), isolated from a *Nocardia* sp., has been shown to exhibit particularly potent activity against *M. tuberculosis* (ATCC 35828), *M. avium* (A26778) and *M. avium* (A25540) with MIC values of ≤ 0.008, 0.06 and 0.25 μg mL^{-1}, respectively.[29-31]



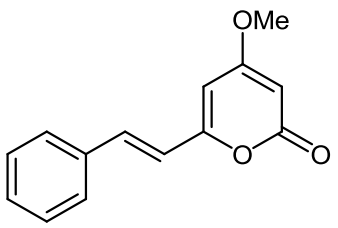
1.12

As part of a screening of various natural and related compounds for activity against *Mycobacterium tuberculosis*, ascididemin (**1.13**), isolated from the Okinawan tunicate *Didemnum* sp.,[32] was identified as a potent inhibitor of *M. tuberculosis* $H_{37}Rv$ with an MIC of 0.1 μg mL^{-1}.[33] The poor solubility and cytotoxicity of this compound, however, make it a poor drug candidate. The synthesis and development of analogues of this potent compound is the subject of research presented in Chapter 2 of this thesis.



1.13

In a screening of antimycobacterial activity of compounds extracted from *Piper sanctum*, Mata *et al.* identified 2-pyranones **1.14** and **1.15** as potential antimycobacterial leads with modest activity against *Mycobacterium tuberculosis* $H_{37}Rv$ of MIC 32 and 4 μg mL^{-1} respectively.[34] While these compounds are not highly active against *M. tuberculosis*, the ease of their synthesis and the associated anti-parasitic activity found in related pyrones makes them interesting targets for development. This compound class is the subject of research presented in Chapter 3 of this thesis.



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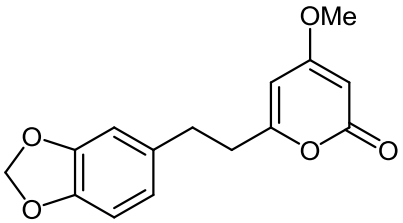


1.15

1.3 Malaria

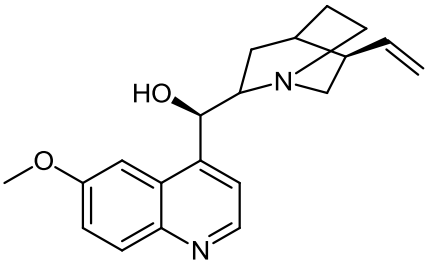
Malaria is a life-threatening disease caused by infection with parasites of the *Plasmodium* genus. These parasites are spread through bites of infected *Anopheles* mosquitoes. The four species of *Plasmodium* that give rise to malaria, are *P. falciparum, P. vivax, P. malariae* and *P. ovale*. The most significant of these species is *P. falciparum*, which is the most deadly and, along with *P. vivax*, is the most common. The WHO estimates that in 2008 there were 247 million cases of malaria and nearly one million deaths as a consequence.[2] *P. vivax* and *P. ovale* have an additional threat in that they lie dormant in the liver of the infected host. Consequently the disease can return again and again. Special treatment for these liver forms is required for complete cure.[2]

One approach to controlling malaria is to limit the mosquito population and their contact with people. Mosquito nets and insecticides remain the standard approach in the control of malarial transmission. Unfortunately resistance amongst the mosquito

population to the insecticides used is becoming more prevalent and a need for an economic and potent mosquito control to slow the spread of disease is becoming increasingly important.[2]

1.3.1 Therapies

The most well known example for the treatment of malaria is quinine (**1.16**), isolated from the bark of the tree *Cinchona succirubra*, which was used for the treatment of malaria for more than three centuries.[35] Quinine was replaced by the structurally related compound chloroquine (**1.17**), which became the drug of choice for the treatment of malaria until resistance to the compound became widespread.[36]



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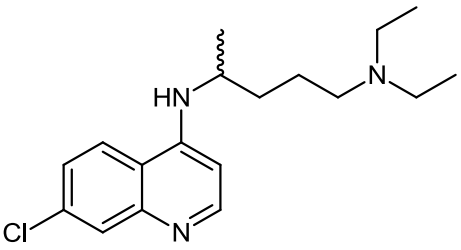
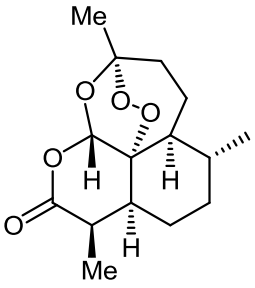


1.17

Chinese herbalists have utilised the *Artemisia annua* shrub for more than 1500 years to treat malaria and in the 1980s the active compound, artemisinin (**1.18**), became used as a medicine. Today, artemisinin is produced via extraction from *Artemisia annua* grown in large scale farming operations. The artemisinin is then derivatised to form the more active compounds, dihydroartemisinin (**1.19**) and artesunate (**1.20**), and used as part of a multi-drug therapy against malaria.[37]



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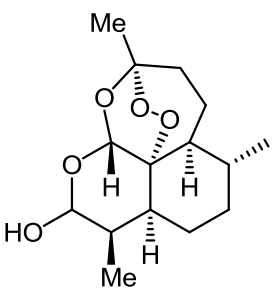

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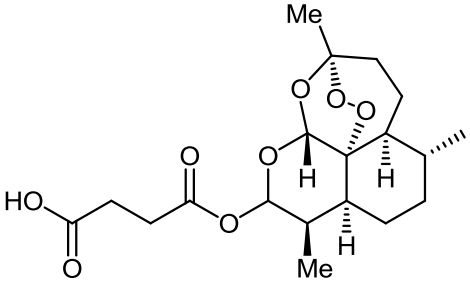


1.20

Currently the best available treatment, particularly for *Plasmodium falciparum* infection, is artemisinin-based combination therapy (ACT). Unfortunately, artemisinin-resistance is becoming more common and is primarily associated with the use of artemisinin mono-therapy rather than ACT.[2,36] Should artemisinin resistance become widespread the consequences could be dire with no alternative anti-malarial agent being ready for deployment in the near-future. The development of new anti-malarials with novel mechanisms of actions are thus of prime importance to the ongoing treatment of this disease.[2]

1.3.2 Anti-malaria drug discovery

Unlike in the case of *Mycobacterium tuberculosis*, the handling of pathogenic *Plasmodium* species is relatively safe and thus primary screening is performed on the target organisms. As *Plasmodium falciparum* represents the most significant health threat the majority of anti-malarial screens focus on activity against this target organism.

The traditional and simplest bioassay analysis involves culturing *Plasmodium falciparum* with human red blood cells in the presence of a known concentration of the compound being evaluated. After incubation for 72 hrs, the samples are stained and the total number of parasites present are counted. Comparison of the control versus the compound doped sample(s) is evaluated and percent inhibition calculated. This relatively simple method requires significant labour and is not suited for high-throughput.[38] To enable greater reproducibility and higher throughput, several alternative assays have been developed. The main development in these assays relates to how the level of plasmodial activity is measured. To move away from a counting by hand, automated methods are used.[38]

Hypoxanthine incorporation assays determine the uptake of tritiated hypoxanthine into a parasitic sample as measured by radioactive emission to indicate the level of parasitic metabolism. This method allows for high throughput but is limited by the handling and costs requirements for radiolabeled substances. A different strategy involves the use of enzyme-linked immunosorbent assay (ELISA) based assays to

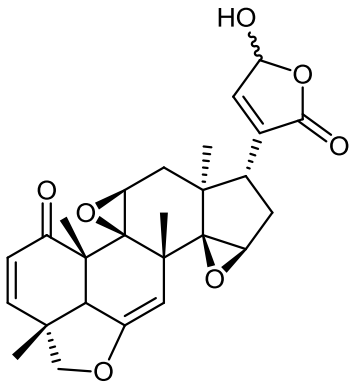
measure plasmodial levels. The histidine-rich protein II (HRP2) method uses protein specific ELISA to measures the level of HRP2 in a sample and in turn the proliferation of *Plasmodium falciparum*. A highly sensitive method for determining drug candidate effects against *P. falciparum* involves the use of modified *P. falciparum*, that expresses a green fluorescent protein, originally from the bioluminescent jellyfish *Aequrea victoria*. Here, the level of plasmodial activity can be spectrophotometrically measured.[38]

These assays help with the discovery of lead anti-malarial compounds which, when combined with a measure of cytotoxicity against mammalian cells, are further analysed for in vivo activity and are then potentially used in clinical trials. More often than not, lead compounds become the subjects of SAR studies in the ongoing development of anti-malarial compounds.

1.3.3 Natural products in anti-malaria drug development

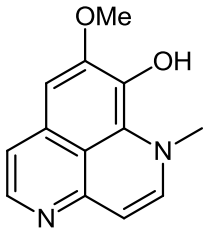
Natural products have been a source of significant anti-malarial agents and include compounds derived from quinine (**1.16**) to artemisinin (**1.18**). Ongoing drug development is primarily driven by the need to treat strains resistant to existing therapies. This relies on the discovery compounds with novel mechanisms of action. Natural products offer a large collection of biologically significant compounds of diverse structural features.[35,36,39-41] A few of these are mentioned below.

In a study investigating the self-medicating habit of wild chimpanzees trichirubine A (**1.21**) was isolated from the leaves of *Trichilia rubescens*.[42] This compound was found to exhibit significant anti-plasmodial activity against *Plasmodium falciparum* with an IC_{50} of 0.3 $\mu g\ mL^{-1}$.[42]



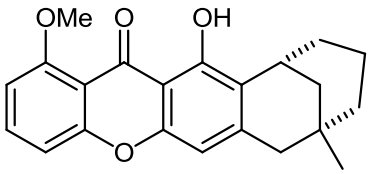
1.21

The marine alkaloid isoaaptamine (**1.22**), isolated from a *Subrites* sp. sponge,[43] has been found to exhibit modest anti-plasmodial activity with an IC_{50} of 0.4 μg mL^{-1} against the W2 clone of *Plasmodium falciparum* and an IC_{50} of 1.1 μg mL^{-1} against the D6 clone.[44]

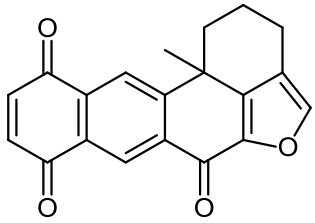


1.22

Investigation of the marine-derived fungus *Chaetomium* sp. led to the isolation of the xanthone chaetoxanthone B (**1.23**). This racemic natural product has shown selective anti-plasmodial activity with an IC_{50} of 0.5 μg mL^{-1} against *Plasmodium falciparum* and an IC_{50} of > 90 μg mL^{-1} against the L6 cell line.[45] A biossay-guided fractionation study, based on a Pfnek-1 inhibition assay, of a marine sponge of the genus *Xestospongia* led to the isolation of xestoquinone (**1.24**). Xestoquinone was found to have moderate antiplasmodial activity (IC_{50} = 3 μM).[46]

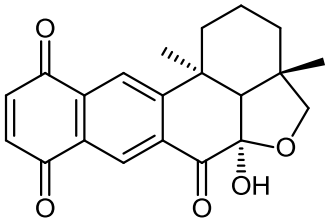


1.23

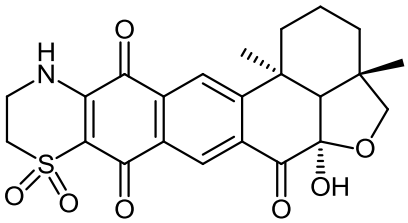


1.24

The related metabolites, alisiaquinone A (**1.25**) and C (**1.26**), isolated from an unidentified New Caledonian sponge, have been found to be antiplasmodial with an IC_{50} of ~ 3 μM and ~ 0.1 μM, respectively.[41]

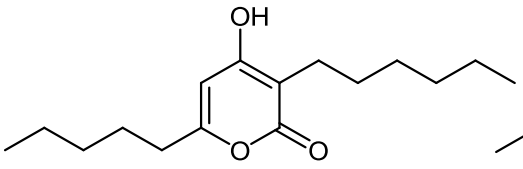


1.25

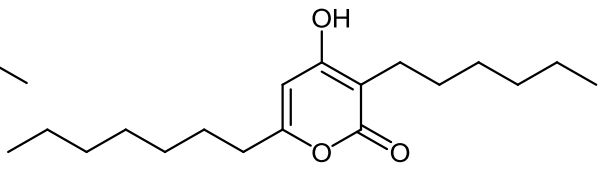


1.26

Marine natural products pseudopyronines A (**1.27**) and B (**1.28**) have been reported to have modest activity against *Plasmodium falciparum* (IC_{50} 15 and 14 μg mL^{-1}, respectively).[47] The 4-O-methyl analogue of pseudopyronine A also exhibited activity against *P. falciparum* (IC_{50} = 3 μg mL^{-1}).[47] The investigation of structure-activity relationships of these compounds is the subject of research presented in Chapter 3.



1.27



1.28

Chapter 2 Asciidemin

2.1 Introduction

The pyridoacridones are a naturally occurring class of alkaloids typically isolated from marine sources. The class is characterised by a tricyclic core (Figure 2.1) typically fused to one or more additional ring systems.

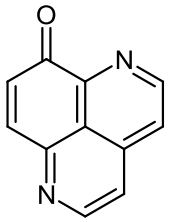
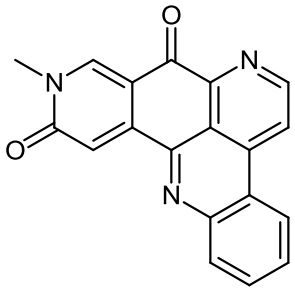


Figure 2.1 The core structure of the pyridoacridones compound class

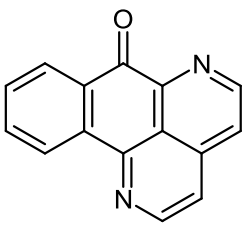
This class of alkaloids are known for their cytotoxicity with many showing strong cytotoxic activity *in vitro*. This activity is generally thought to be a consequence of their potential as DNA intercalators, suggested by their planar core structure. Due to this cytotoxic nature, extensive studies on the antitumor activities of pyridoacridones have been reported.[48-50] In addition, various other biological activities have been found for members of this class of alkaloid including antibacterial, antifungal and antiviral properties.[49]



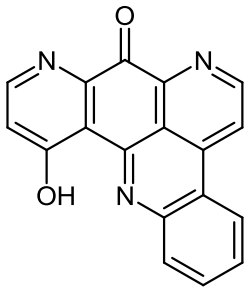
2.1

Amphimedine (**2.1**), the first reported pyridoacridone,[51] was isolated from an *Amphimedon* sp. sea sponge. It has been reported to have cytotoxicity against p388 murine leukemia cell lines at 0.3-0.4 mg mL^{-1}.[52] This level of cytotoxicity, however, has been reported not to be useful for the development of anti-cancer agents.[53]

Sampangine (**2.2**), isolated from the plant *Cananga odorata*,[54] is one of the simplest pyridoacridones of this class, and is reported to posess potent anti-microbial activity against *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus fumigatus* and *Mycobacterium intracellulare*.[55] The marine alkaloid meridine (**2.3**) has been shown to have considerable antifungal activity.[56]



2.2



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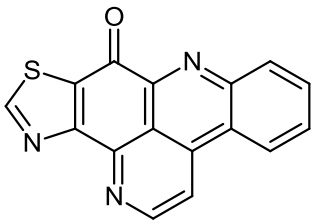

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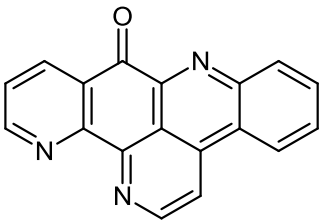
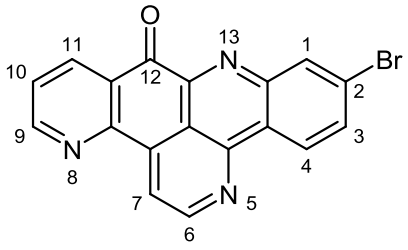


2.5

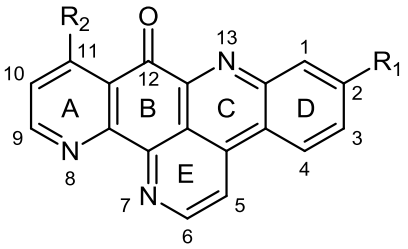
Kuanoniamine A (**2.4**), isolated from a marine tunicate,[57] is an example of a sulfur containing pyridoacridone alkaloid which exhibits potent cytotoxicity with an IC_{50} of 0.03 μM against the human colon tumour cell line HCT-ll6.[58] The structurally related marine alkaloid ascididemin (**2.5**) also exhibits numerous biological activities and will be discussed in greater detail in the following sections.

2.1.1 Ascididemin: Discovery and biological activity

In 1987 Schmitz *et al*. reported the structure of 2-bromoleptoclinidinone **2.6** from an ascidian tentatively identified as *Leptoclinides* species.[59] The alkaloid was shown to have mild cytotoxic properties with an ED_{50} towards the PS lymphocytic leukemia cell line of 0.4 μg mL^{-1}. The fused pentacyclic structure was considered a very rare motif for marine organisms with only two other examples reported at that time.



2.6



2.5 $R_1=R_2=H$
2.7 $R_1=Br$, $R_2=H$
2.8 $R_1=H$, $R_2=OH$

In 1988 the marine alkaloid ascididemin **2.5** was isolated by Kobayashi *et al*.,[32] from the Okinawan tunicate *Didemnum* sp., and found to be cytotoxic with an IC_{50} of 0.39 μg mL^{-1} against L1210 murine leukemia cells *in vitro*. The reported structure prompted a structural revision of 2-bromoleptoclinidinone to 2-bromoascididemin **2.7**.[60] This structure had originally been discounted due to the apparent lack of Fe (II) chelation expected with nitrogen atoms at the 7- and 8- positions. 1,10-Phenanthroline changes colour to deep red upon the addition of Fe(II) whereas 2-bromoascididemin did not give a visible colour change and thus was believed to be non-chelating towards Fe(II).[60] The lack of a visible colour change is presumably due to the extended conjugation of the system which would lead to a non-visible (UV) change in absorbance on chelation.

A third member of the ascididemin series, 11-hydroxyascididemin **2.8**, isolated from *Leptoclinides* sp.[61,62] was reported in 1990. This molecule was also found to exhibit cytotoxicity towards cultures of murine leukemia cells (P388) with an IC_{50} of 0.3-0.4 μg mL^{-1}, later seperately reported as 2.3 μM.[63] In 1994 a meridine-like alkaloid, cystodamine, was reported but the structure was subsequently revised to 11-hydroxyascididemin.[64,65]

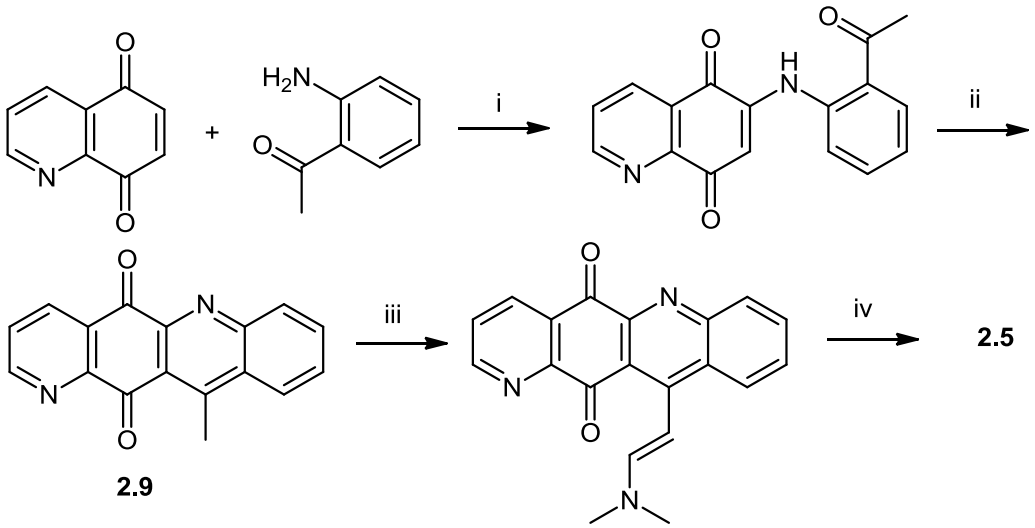
Ascididemin **2.5** exhibits a range of different biological activities, including the ability to inhibit the DNA processing enzyme topoisomerase II at a concentration of 30 μM.[62] In 1995 Copp *et al*. reported additional cytotoxicity data and antimicrobial activities for ascididemin as part of a preliminary structure-activity study.[66] Cytotoxicity towards mouse leukemia (P388), human colon (HCT-116) and human breast (MCF7) tumour cell lines was reported with an IC_{50} of 0.4, 0.3 and 0.3 μM, respectively. Selective cytotoxicity towards the DNA double strand break repair deficient Chinese Hamster Ovary (CHO) cell line xrs-6 versus the repair competent wild type CHO cell line BR1 was reported.[66] Toxicity towards non-malignant African Green Monkey kidney cell-line BSC-1, bacteria *Bacillus subtilis* (Gram-positive) and *Eschericha coli* (Gram-negative) and fungi *Candida albicans* and *Cladisporium resinae* has also been reported.[66] In 1995 Duncan *et al*. reported ascididemin as an example of a hit, yielded by a newly developed high-throughput antimycobacterial screen, with an IC_{90} of 0.21 μg mL^{-1} and a MIC of 0.25 μg mL^{-1}.[67] Further to this, additional biological data has been progressively reported: a lack of antiviral potential,[58,63] antimicrobial activity,[63,68] antiparasitic activity,[49,69] selective topoisomerase II inhibitory activity,[70] anti-tumour activity,[32,48,58,68,71-75] telomerase inhibitory[76] and antitubercular activity.[33,77] By way of comparison, 2-bromoascididemin **2.7** has been shown to have antitumour activity with a similar profile to ascididemin,[48] and antiparasitic activity,[69] but no data is available for other biological activities. Similarly, 11-hydroxyascididemin showed antitumour activity but with a significantly reduced (~10 fold) potency compared to the other two natural products.[48] In contrast to ascididemin, 11-hydroxyascididemin exhibits moderate activity against both DNA (Herpes simplex (ATCC VR 733)) and RNA (Polio virus 1 (Pfizer vaccine strain) viruses but no protective activity against HIV.[63]

Several studies have explored the mechanism of action of ascididemin. In addition to cytotoxicity and topoisomerase II inhibition, ascididemin was shown to have the ability to induce reductive DNA cleavage.[78] Dirsch *et al*. demonstrated ascididemin causes mitrochondrial dysfunction leading to apoptosis.[79] DNA microarray data has shown that ascididemin has antimycobacterial activity that is partially dependant on iron scavenging but also has additional activity not present in other iron scavenging

molecules.[77] More recently reports of ascididemin as a chemical defence for the ascidian *Cystodytes* sp. have been published.[80-82] López-Legentil *et al*. have shown that ascididemin deters predation of the ascidian. The addition of sulfuric acid eliminated the deterrent activity, which was believed to be due to protonation of the alkaloid.

2.1.2 Syntheses of pyridoacridone ascididemin

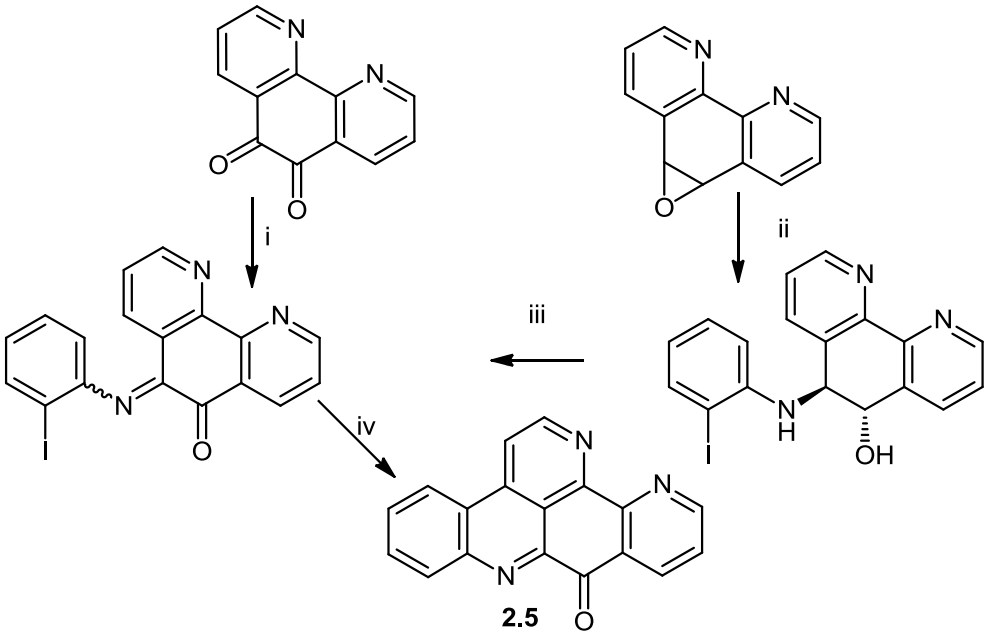
In 1989 Bracher reported the first synthesis of ascididemin.[83] His method proceeded by the oxidative amination of quinoline-5,8-quinone with *o*-aminoacetophenone in the presence of cerium trichloride and air (Scheme 2.1). The diaryl amine adduct was cyclised with concentrated sulphuric acid in glacial acetic acid to give rings tetracyle **2.9**. Ring E was furnished via enamine formation with dimethylformamide diethyl acetal followed by reflux with ammonium chloride in acetic acid to give ascididemin (**2.5**).



Scheme 2.1 The first published synthesis of ascididemin.[83]
Reagents and conditions: i) $CeCl_3.7H_2O$, CH_3CH_2OH, air, 20ºC, 16 h (78%); ii) conc. H_2SO_4:AcOH(1:10), reflux, 10 min (94%); iii) DMF-DEA, DMF, 120ºC, 1 h; iv) NH_4Cl, AcOH, reflux, 1 h (59%).

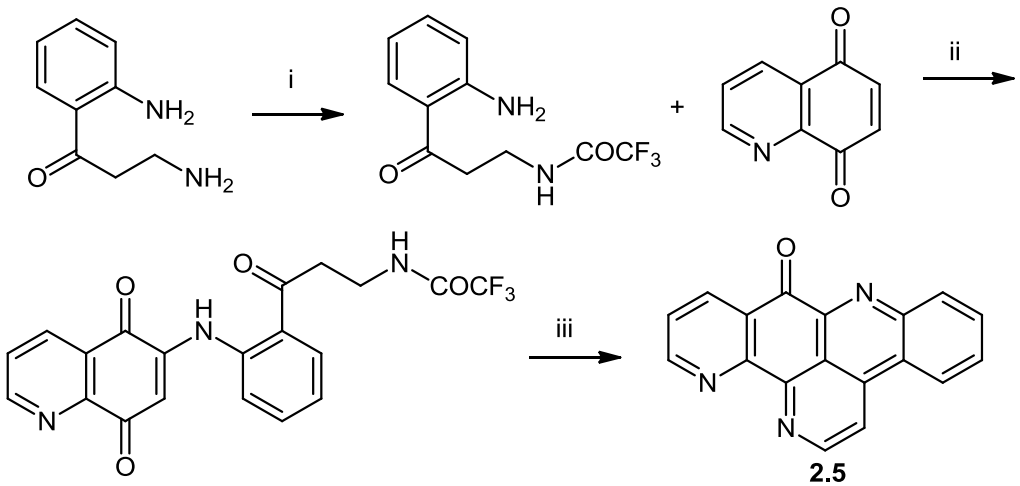
A second route was reported in 1990.[84,85] This route (Scheme 2.2) starts with rings A, B and E from either of two different starting materials. Introduction of ring D was achieved by a novel aza Wadsworth-Emmons reaction [Scheme 2.2, step (i)] however, due to low yields an alternative route was favoured. This second approach starts with epoxide ring opening followed by oxidation of the resultant alcohol (Scheme 2.2,

steps (ii) and (iii)). Finally photocyclisation was used to close ring C and give ascididemin.



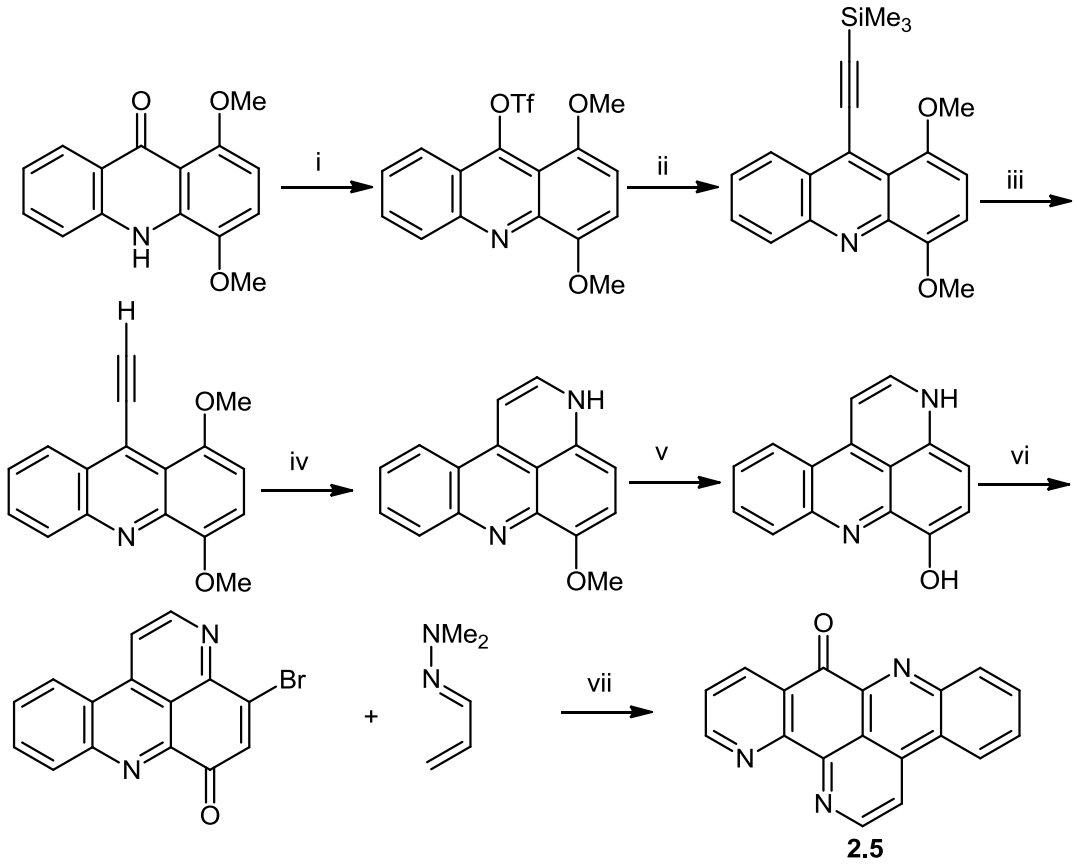
Scheme 2.2 The second synthesis of ascididemin.[84,85]
Reagents and conditions: i) 2-$IC_6H_4NHP(O)(OEt)_2$, NaH, glyme (saturated polyethers); ii) 2-$IC_6H_4NH_2$, Et_3Al, CH_2Cl_2; iii) $BaMnO_4$, CH_2Cl_2; iv) hv, H_2SO_4.

In 1993, a biomimetic synthesis of ascididemin was reported (Scheme 2.3).[86] As with Bracher's methodology, this method used oxidative amination of quinoline-5,8-quinone in the presence of cerium trichloride and air, but this time using a more functionalised amine. The resultant adduct could be converted to ascididemin in two steps; an annulation using base or acid to form two rings, followed by oxidation with air.



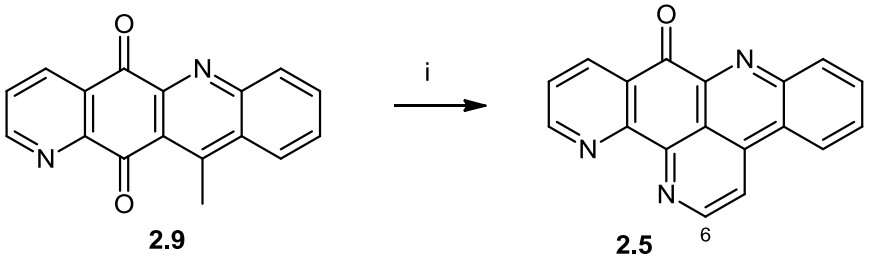
Scheme 2.3 Biomimetic synthesis of ascididemin.[86]
Reagents and conditions: i) CF_3CO_2Et, MeOH, r.t.; ii) $CeCl_3$, r.t.; iii) (1) NH_4OH or 4N HCl, (2) O_2.

In 2000 a significantly different synthesis of ascididemin was reported.[71] This method started with the introduction of a triflate group followed by palladium cross-coupling with a protected acetylene group. In turn the acetylene group was deprotected and annulated to form ring E. Subsequent demethylation and bromination produced a regiospecific dienophile which finally underwent hetero Diels-Alder cycloaddition to give ascididemin **2.5** (Scheme 2.4).



Scheme 2.4 Synthesis of ascididemin.[71]
Reagents and conditions: i) Tf_2O, DMAP, 2,6-lutidine, 0ºC to r.t, 87%.; ii) HCCTMS, $Pd(PPh_3)_4$, THF, iPr_2NEt, reflux, 98%; iii) KF, MeOH, reflux, 97%; iv) $NaN(CHO)_2$, DMF, reflux, 55%; v) BBr_3, CH_2Cl_2, -78ºC to r.t.; vi) NBS, DMF, 0ºC, 53%, vii) 10 Kbar, 80ºC, MeCN, 21%

Copp *et al.* reported an alternative annulation of tetracycle **2.9**, generated via Bracher's methodology, utilising paraformaldehyde (Scheme 2.5).[58] This method provides for the facile introduction of substituents at the six position via the use of alternative aldehydes.



2.9 **2.5**

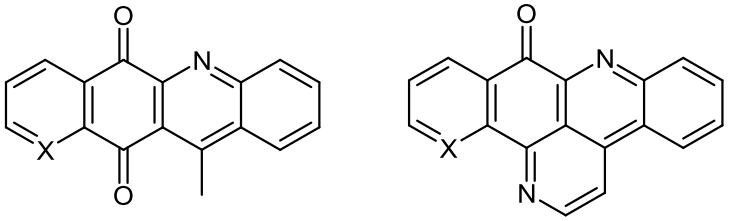
Scheme 2.5 Ring E formation step to give ascididemin.[58]
Reagents and Conditions: i) $(CH_2O)_n$, NH_4Cl, AcOH

Each of the described synthetic approaches to ascididemin offers the ability to introduce different R groups at different positions of the molecule. For analogues varying at the 6-position, the last approach (Scheme 2.5) offers the greatest flexibility and simplicity.

2.1.3 Previous SAR studies of ascididemin

Several studies on the structure-activity of ascididemin have been reported. These studies have primarily focused on the antitumour activity of this class of compounds, with some studies investigating antimicrobial, and more recently, antimycobacterial activity.

In 1995 Copp *et al*. reported numerous assay results for ascididemin, 8-deaza-ascididemin **2.11** and their synthetic precursors **2.9** and **2.10.**



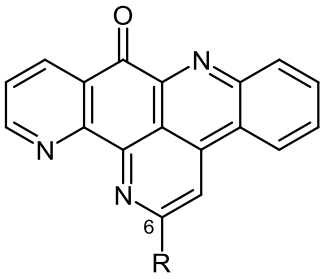
2.9 X=N
2.10 X=CH

2.5 X=N
2.11 X=CH

The synthetic precursors had little of the biological activity exhibited by ascididemin which indicated the importance of the E ring to the observed biological activity. 8-Deaza-ascididemin (**2.11**) exhibited reduced cytotoxicity compared to ascididemin with a four fold decrease in activity towards the mouse leukemia tumour cell line (P388) and a 1000+ fold drop in activity against human colon (HCT116) and human

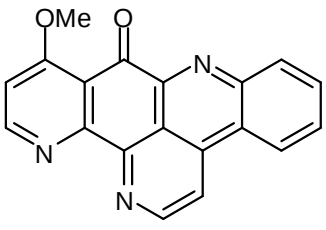
breast (MCF7) tumour cell lines. This result indicated the presence of multiple mechanisms of action in mammalian cell lines. The antimicrobial activity was found to be modulated by the presence or absence of N-8 with some antimicrobial activities requiring the presence of the nitrogen and others requiring the absence.[66]

These results were expanded upon with a report of a more expansive library of analogues.[68] This library covered synthetic precursors, simplified structures (one or more of the rings removed) and six-substituted ascididemin analogues. The biology reported covered antiviral, antimicrobial and antitumour activity. Analogues substituted at the six position possessed reduced cytotoxicity and antimicrobial activity.



2.12 R = CH_3
2.13 R = C_6H_5
2.14 R = CH=CH-C_6H_5

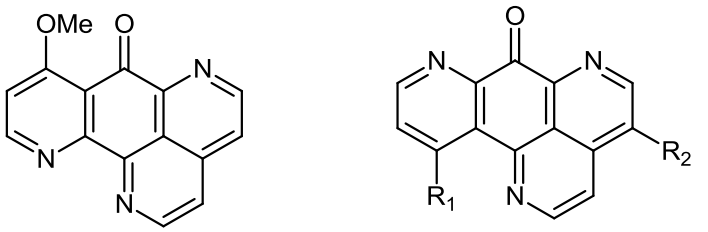
In 2002 Delfourne *et al*. reported the first of several structure-activity studies into the *in vitro* antitumour activity of ascidemin.[73] In this study various substituents on the core ascididemin structure were examined. 11-Hydroxyascididemin and the methylated analogue **2.15** showed greatly reduced antitumour activity. The other analogues tested showed comparable or greater activity with in some cases, an average of a 10-fold increase in antitumour activity.



2.15

In a separate study, Delfourne *et al*. investigated tetracyclic structures based on ascididemin and meridine (examples Figure 2.2).[74] These simplified structures primarily showed less antitumour activity than ascididemin with the exception of two

analogues **2.17** and **2.18** showing significantly improved activity. In a further study additional compounds with these core structures were reported.[75] While most of the examples given were of comparable activity to those previously published, a small number had greatly increased cytotoxicity. For example, **2.19** was reported to have an IC_{50} of 0.4 nM towards two tumour cell lines (the T-24 and PC-3 cell lines).[75]

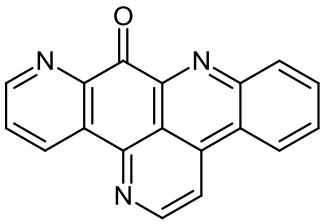


2.16

2.17 R_1=H, R_2=OMe
2.18 R_1=R_2=OMe
2.19 R_1=H, R_2=piperidyl

Figure 2.2 Tetracycles examined in SAR study

In 2003 an investigation of substituted 8-deaza-11-aza-ascididemin **2.20** established that it and analogues exhibited comparable antitumour activity to that observed for ascididemin.[72]



2.20 8-deaza-11-aza-ascididemin

Investigation of the antimycobacterial properties of the ascididemin class of pyridoacridones has been reported (Figure 2.3).[33] The structures investigated were a mix of synthetic precursors and related analogues. It was demonstrated that the antitubercular activity of this class of compound was related to, but not dependant upon, cytotoxicity. This was shown through the use of a selectively index, the ratio of IC_{50} of compounds towards the non-malignant VERO cell line to the MIC of the compounds against *M. tuberculosis*. This index was shown to have considerable variation, with one compound **2.22** having a selectivity index of 15.1 and another

compound **2.21** having an index of <0.08. The difference in these two structures is the presence (or absence) of a nitrogen atom.

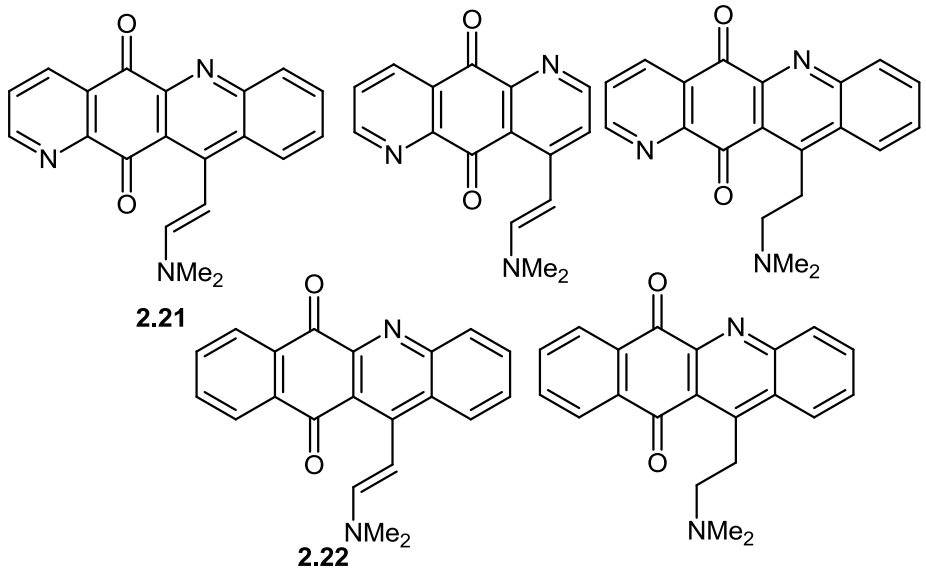


Figure 2.3 Examples of ascididemin precursors and analogues evaluated against tuberculosis[33]

2.1.4 Project aims

The natural product ascididemin has been shown to be a highly active antitubercular agent. However, due to its toxicity to normal human cells and its low water solubility ascididemin is unsuitable for use as a therapeutic agent.

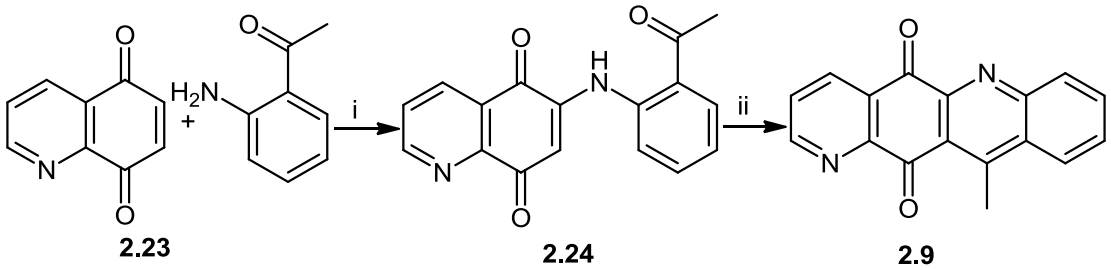
The aim of this project was the preparation of a library of novel analogues of ascididemin with sterically bulky substituents at the 6-position. The nature of these substituents were to be developed via an iterative sequence of synthesis and biological testing against tuberculosis *in vitro*, with the ultimate target being to prepare analogues with enhanced solubility relative to the parent molecule, and greatly diminished cytotoxicity. In addition, the equivalent 6-substituted-8-deaza-ascididemin analogues were also targets for preparation. These examples were prepared concurrently to the appropriate 6-substituted-ascididemin and evaluated for their biological activity. Previous studies have shown marked variation in biological activity in molecules differing at just this site. Of interest is the reported lowered cytotoxicity, with respect to the 8-aza compounds, of these molecules.

The preparation and biological evaluation of such compounds will allow a conclusion to be drawn as to whether the ascididemin skeleton has the potential for further development as an anti-tuberculosis agent.

2.2 Results and Discussion

2.2.1 Chemistry

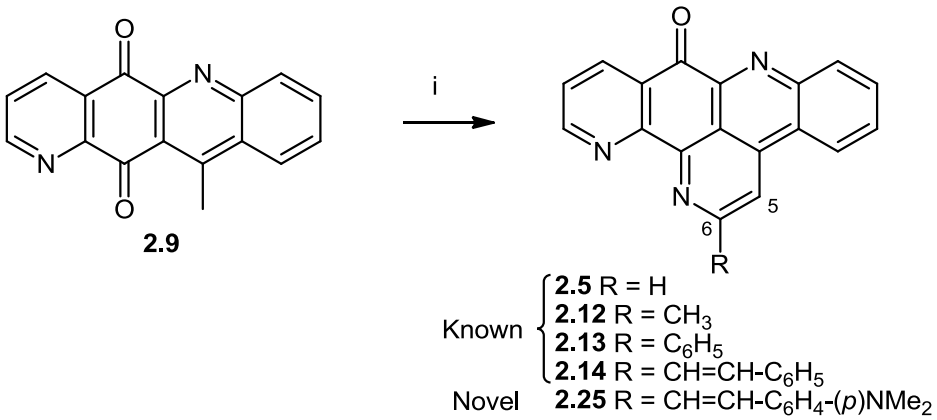
Following the literature procedure reported by Bracher, 11-methylpyrido[2,3-*b*]acridine-5,12-dione **2.9** was prepared.[83] Here, quinoline-5,8-dione (**2.23**), cerium trichloride heptahydrate and 2'-amino acetophenone were stirred in ethanol for 18 hrs at which time the crystalline product was filtered from the reaction mixture and washed with ice-cold ethanol. The product **2.24** was characterised by ^{1}H and ^{13}C NMR spectroscopy as well as mass spectrometry. This characterisation was consistent with the expected structure and with data previously published.[87]



Scheme 2.6 Preparation of **2.9**, Bracher's methology.
Reagents and Conditions: i) $CeCl_3.7H_2O$, EtOH, air, 20ºC, 16 h (78%); ii) conc.-H_2SO_4-AcOH (1:10), reflux, 10 min (94%).

Cyclisation of adduct **2.24** proceeded smoothly via heating at reflux in concentrated sulphuric acid:acetic acid (1:10) for 20 min. Once cool, the reaction mixture was suitably diluted and neutralised with concentrated aqueous ammonia. The target tetracycle **2.9** was obtained by extraction of the resulting suspension with dichloromethane, which in turn was washed with water, followed by evaporation. Characterisation of the crude product by NMR spectroscopy and mass spectrometry confirmed the expected structure and was consistent with previously published data.[83,88]

Utilising the general methodology developed by Lindsay *et al*.[58] the preparation of known 6-substituted ascididemin analogues (**2.5**, **2.12**, **2.13** and **2.14**) was undertaken, as shown in Scheme 2.7.



Scheme 2.7 General procedure for the preparation of 6- substituted ascididemin analogues. Reagents and conditions: i) RCHO, NH_4Cl, AcOH, reflux.

The reaction of paraformaldehyde, tetracycle **2.9** and ammonium chloride was conducted in refluxing acetic acid under a nitrogen atmosphere. Upon cooling the reaction mixture was diluted with water and neutralised with concentrated aqueous ammonia. The product was extracted into dichloromethane, partitioned with water and dried in vacuo. Precipitation of crude material from dichloromethane with methanol gave ascididemin **2.5**. The ^{1}H NMR spectrum clearly showed the loss of methyl protons (δ_H 3.33, 3H, s) and the introduction of two additional aromatic protons, two doublets (J = 5.8 Hz) at δ_H 9.30 and 8.57, consistent with the introduction of protons at positions 5 and 6. In addition to the NMR data, the molecular ion present in the EI mass spectrum (*m/*z 283) was consistent with the formation of ascididemin. Characterisation of ascididemin by melting point, ^{1}H NMR and mass spectrometry was in full agreement with published data.[32,60]

6-Methyl-ascididemin **2.12** was prepared from **2.9** in a similar fashion by reflux with acetaldehyde and ammonium chloride in acetic acid under a nitrogen atmosphere. Following water-dichloromethane partitioning, the crude material was purified first by silica gel flash chromatography and finally by precipitation from dichloromethane with methanol. Spectral data of **2.12** confirmed the desired structure had been

produced. The EI mass spectrum contained the expected molecular ion of *m/z* 297. The ^{1}H NMR spectrum of **2.12** shows the expected differences from that of **2.5** with a methyl singlet now present at 3.08 ppm, the loss of a doublet for H-6 and the presence of a singlet at 8.42 ppm (H-5) in place of the doublet found for **2.5**. The melting point, HREIMS and ^{1}H NMR were all in agreement with published data.[68]

Preparation of 6-phenyl-ascididemin **2.13** from benzaldehyde, **2.9** and ammonium chloride was carried out by reflux in acetic acid under a nitrogen atmosphere. Water-dichloromethane partitioning gave crude material which was purified by silica gel flash chromatography followed by precipitation from dichloromethane with methanol. The ^{1}H NMR data of **2.13** was consistent with the formation of the desired product and the expected molecular ion (*m/z* 359) was observed in the EI mass spectrum. The ^{1}H NMR spectrum showed the expected variation in the proton chemical shifts associated with the tetracycle moiety compared to **2.9**. In addition, the expected loss of the methyl singlet at δ_H 3.33 ppm was observed. As with **2.12,** an aromatic singlet associated with H-5 was observed, this time at δ_H 8.89 ppm. A doublet and a triplet each with an integral of 2H, along with a triplet integrating for 1H were observed at δ_H 8.36, 7.59, and 7.53 ppm, respectively, as expected for a mono-substituted phenyl ring. The melting point, HREIMS and ^{1}H NMR were consistent with published data.[68]

6-*E*-Styryl-ascididemin **2.14** was prepared in turn from cinnamaldehyde and **2.9** refluxed in acetic acid under a nitrogen atmosphere, in the presence of ammonium chloride. The crude material was purified by silica gel flash chromatography followed by precipitation from dichloromethane with methanol. Spectral data for **2.14** confirmed the formation of the desired product. The expected molecular ion of *m/z* 386 was detected in the FAB mass spectrum. The ^{1}H NMR spectrum was consistent with that observed for the phenyl analogue **2.13** with the addition two doublets, δ_H 8.03 and 7.62 ppm, each with a large coupling constant of 16 Hz consistent with the presence of an *E* alkene. The ^{13}C NMR spectrum showed the expected number of carbon environments. The melting point, mass spectrum, ^{1}H and ^{13}C NMR spectra were all consistent with published data.[68]

6-(4-Dimethylamino-*E*-styryl)-ascididemin **2.25** was prepared from **2.9** and 4-dimethylaminocinnamaldehyde. As with previous examples the reaction was carried out in acetic acid in the presence of ammonium chloride under reflux conditions and a nitrogen atmosphere. Purification of the crude material was accomplished using silica gel flash chromatography followed by precipitation from dichloromethane with *n*-hexane. Spectral data confirmed **2.25** to be the desired product. The presence of the molecular ion *m/z* 429 in the FAB mass spectrum was consistent with the expected product. The ^{1}H NMR spectrum of **2.25** was comparable to that for 6-*E*-styryl-ascididemin **2.14**. An upfield change in chemical shift for H-18, from δ_H 7.43 ppm to δ_H 6.66 ppm, was observed and is explained by the proximity to the dimethylamino group. In addition, the absence of a proton resonance corresponding to position 19 and the presence of a singlet, δ_H 3.01 ppm, 6H, was observed indicating the presence of the dimethylamino group at position 19. The ^{13}C NMR spectum for **2.25** showed the expected number of carbon environments and with the aid of 2D NMR studies, consisting of HSQC and HMBC experiments, was fully assigned along with the ^{1}H NMR data. Critically the ^{1}H NMR signals for H-5 and H-21 as well as the ^{13}C NMR signal for C-12 were sufficiently distinct to be readily assigned and used as starting points for full assignment. Selected HMBC correlations demonstrating how the assignment was made are shown in Figure 2.4.

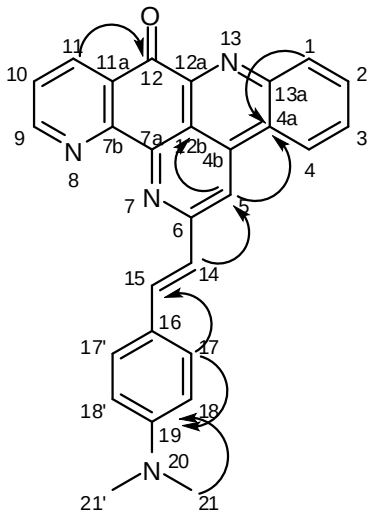
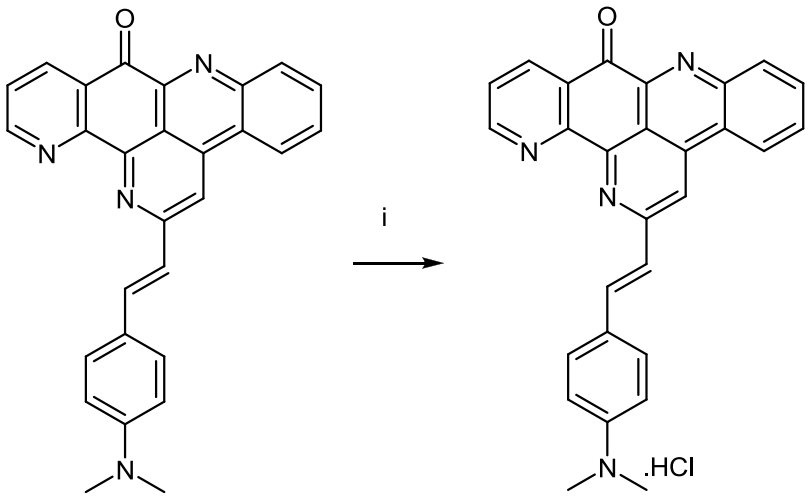


Figure 2.4 Selected HMBC correlations observed for **2.25**

To increase water solubility HCl salt formation of **2.25** was undertaken by the precipitation of a dichloromethane solution of **2.25** with ethereal hydrogen chloride to give **2.26**. The resultant orange solid was filtered and washed with diethyl ether. The product was air dried followed by brief (1 min) exposure to high vacuum. Longer exposure to high vacuum resulted in reversion to the free base as evidenced by a colour change from orange to green and confirmed by ^{1}H NMR spectroscopy. Formation of the product was indicated firstly by a distinct change in solubility characteristics. The acidified product **2.26** was insoluble in dichloromethane but was freely soluble in water while the starting material was relatively soluble in dichloromethane and insoluble in water. The ^{1}H NMR spectrum, taken in d_6-DMSO, was sufficiently broadened to make assignment exceptionally difficult. The peak shifts and integrals however were consistent with those expected for the desired product. Further confirmation of the formation of salt **2.26** was achieved by the regeneration of the starting material in the free base form. A small amount of the hydrochloride salt was suspended in dichloromethane, and upon dropwise addition of concentrated ammonia the solid dissolved producing a dark red solution. After drying in vacuo the product solubility matched that of the free base **2.25** and ^{1}H NMR spectrum matched perfectly that of previously recorded spectra of **2.25**.

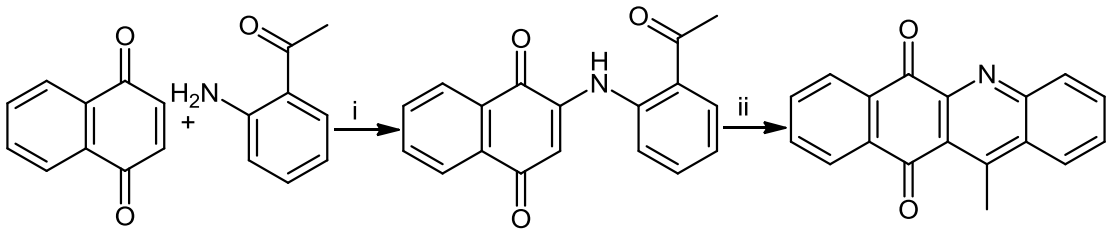


2.25 **2.26**

Scheme 2.8 The preparation of hydrochloride salt **2.26**.
Conditions: i) CH_2Cl_2, ethereal HCl.

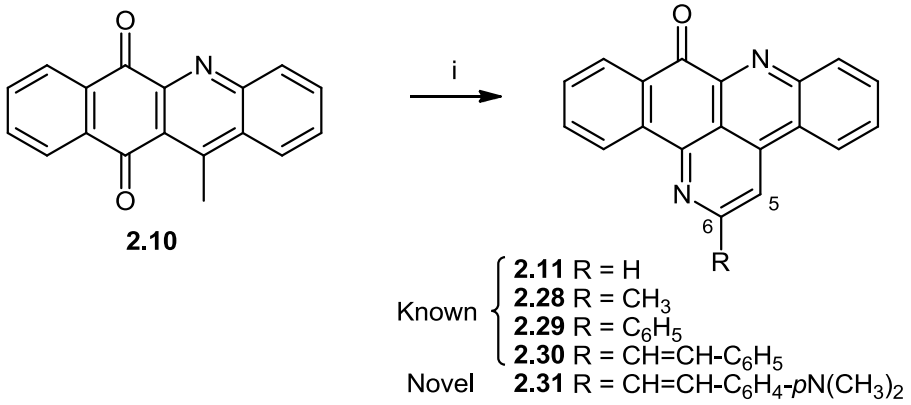
8-Deaza analogues of ascididemin and 6-substituted ascididemin represented a desirable expansion of the set of target compounds. Following literature procedures, tetracycle **2.10** was prepared as a central starting material for this class of

compound.[55,87] Here, 2-[(2-acetylphenyl)amino]-1,4-napthalenedione **2.27** was prepared following a literature procedure analogous to that used to prepare **2.24**.[55] A solution of 1,4-napthoquinone, cerium trichloride heptahydrate and 2'-amino-acetophenone in ethanol was stirred for 48 hrs. The suspended red crystalline product was filtered and washed with ice-cold ethanol. ^{1}H and ^{13}C NMR spectra along with high resolution mass spectrometry data were as expected for the desired product and compared favourably with literature data.[55]



Scheme 2.9 Preparation of **2.10**
Conditions: i) $CeCl_3.7H_2O$, EtOH, air, 20ºC, 16 h (61%); ii) conc.-H_2SO_4-AcOH 1:10), reflux, 40 min (54%).

11-Methylbenz[*b*]acridine-5,12-dione **2.10** was in turn prepared following a literature procedure.[55] Adduct **2.27** was cyclised by heating in a concentrated sulphuric/acetic acid mixture (1:9) at reflux for 40 mins. Neutralisation with concentrated ammonia and extraction with dichloromethane gave the crude product as a tan solid. The crude material was sufficiently pure by ^{1}H NMR spectroscopy to be used without additional purification. High resolution EI mass spectrometry data for **2.10** predicted the expected molecular formula for the molecular ion while the ^{1}H and ^{13}C NMR spectra showed signals containing the appropriate number of chemical environments and chemical shifts as previously reported.[55]



Scheme 2.10 Preparation of deaza-ascididemin analogues.
Reagents and conditions: i) RCHO, NH_4Cl, AcOH, reflux.

Tetracycle **2.10** and paraformaldehyde were reacted by heating at reflux in acetic acid to give 8-deaza-ascididemin **2.11** (Scheme 2.10). Neutralisation with concentrated ammonia and extraction with dichloromethane gave crude material which was purified by flash chromatography. The ^{1}H NMR data for **2.11** was distinct from starting material **2.10** with the expected loss of the methyl singlet resonance (δ_H 3.24, 3H, s) and the introduction of two aromatic signals (δ_H 8.96, 1H, d, J = 5.7 Hz; δ_H 8.29, 1H, d, J = 5.7 Hz) expected for protons present at positions 5 and 6. Furthermore the ^{13}C NMR data, the high resolution mass spectrometry data and the melting point all confirmed **2.11** as the desired product and matched previously reported data.[55]

8-Deaza-6-methyl-ascididemin **2.28** was prepared in a similar fashion by the reaction of **2.10,** acetaldehyde and ammonium chloride in acetic acid under nitrogen. After workup the product was purified by silica flash chromatography followed by precipitation from dichloromethane with methanol. The ^{1}H and ^{13}C NMR data observed for **2.28** were very similar to those observed for **2.11** with significant changes to the signals associated with position 5 (from δ_H 8.29, 1H, d, J = 5.7 Hz; δ_C 115.3, to δ_H 8.15, 1H, s; δ_C 113.9) and 6 (from δ_H 8.96, 1H, d, J = 5.7 Hz; δ_C 148.8, to δ_C 158.7), as well as the introduction of signals as expected for an additional methyl group (δ_H 2.87, 3H, s; δ_C 25.5). Mass spectrometry gave the anticipated molecular ion at *m/z* 296 and the molecular formula for the product ($C_{20}H_{12}N_2O$) under high resolution analysis. The NMR data, HREIMS and melting point were all in agreement with the previously reported data.[87]

The annulation of tetracycle (**2.10**) with benzaldehyde and ammonium chloride occurred in refluxing acetic acid under nitrogen to give 8-deaza-6-phenyl-ascididemin **2.29**. The crude material was purified by silica flash chromatography followed by precipitation from dichloromethane with methanol. The NMR data resembled that of **2.28** with differences arising from the presence of a phenyl group instead of a methyl group at the 6- position. These differences included the loss of the methyl signal (δ_H 2.87, 3H, s; δ_C 25.5) and the introduction of three additional aromatic environments (δ_H 8.32, 2H, dd, J = 8.1, 1.4 Hz; δ_C 127.5. δ_H 7.61-7.50, 3H, m; δ_C 129.0, 130.0. δ_C 138.6) due to the presence of an additional benzene ring. The NMR

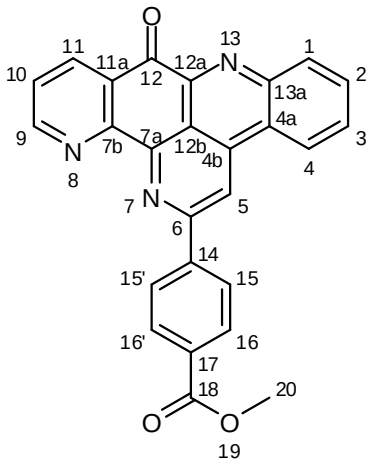
data, mass spectrum and the melting point were all in agreement with that of a previously reported data of **2.29**.[87]

Following the general procedure shown in Scheme 2.10, 8-deaza-6-*E*-styryl-ascididemin **2.30** was prepared via the reaction of tetracycle **2.10**, cinnamaldehyde and ammonium chloride in refluxing acetic acid under nitrogen. Purification of the crude material was undertaken by silica flash chromatography. The ^{1}H and ^{13}C NMR data of the product was similar to that of **2.29** with minor variation in chemical shifts and the addition of two signals (δ_H 7.96, 1H, d, J = 15.7 Hz; δ_C 135.4. δ_H 7.24, 1H, d, J = 15.8 Hz; δ_C 127.1) corresponding to the alkene group. Full characterisation by NMR, mass spectrometry and melting point agreed with that of previously recorded data.[87]

8-Deaza-6-(4-dimethylamino-*E*-styryl)-ascididemin **2.31** was similarly prepared from tetracycle **2.10**, 4-dimethylaminocinnamaldehyde and ammonium chloride in refluxing acetic acid under a nitrogen atmosphere. The crude material was purified using silica flash chromatography followed by precipitation from dichloromethane with hexane. The ^{1}H and ^{13}C NMR spectra for **2.31** possessed the signals for the main pentacyclic moiety as observed in the previous examples, most closely following the equivalent signals of **2.30**. For the remainder of the molecule there were observed resonances consistent with the presence of a highly desheilded *E* alkene (δ_H 8.03 and 7.17, d, J = 15.6 Hz, δ_C 136.0 and 122.7), a *para* disubstituted phenyl moiety (δ_H 7.57, 2H, d, J = 8.8 Hz, δ_C 128.9; δ_H 6.74, 2H, d, J = 8.8 Hz, H-18, δ_C 112.1; δ_C 150.9; δ_C 124.4) and a dimethylamino group (δ_H 3.04, 6H, s, δ_C 40.3). With aid of 2D NMR experiments (COSY, HSQC, HMBC), the ^{1}H and ^{13}C NMR data were assigned; the HMBC data readily linked the dimethylamino to the 1,4-disubstituted phenyl group, with protons on the 21,21'- and 17,17'- positions correlating to carbon C-19. Protons on the 17,17'- position also correlating to carbon C-15 of the *E* alkene. The *E* alkene protons were similarly correlated to the main pentacyclic moiety which was assigned in the same fashion as previous examples. The EI mass spectrum for **2.31** contained the expected molecular ion at *m/z* 427 further confirming the desired product's formation.

As in the case of dimethylamino ascididemin analogue **2.25**, attempts were made to prepare a water soluble salt of **2.31**. Precipitation of **2.25** from dichloromethane with ethereal hydrogen chloride generated a purple solid. This solid dissolved in water only to precipitate out of solution moments later. The supposed salt was not further evaluated.

In order to access more water soluble compounds it was desirable to introduce a protected functional group onto the ascididemin scafford that could then be readily used to introduce varied solubilising moities. One classical approach is to introduce either amino or carboxylic acid functional groups which can then be used in the formation of an amide linkage via a multitude of procedures. The first approach attempted was the introduction of a carboxylic acid functional group. Methyl ester **2.32** was therefore a logical choice of target.

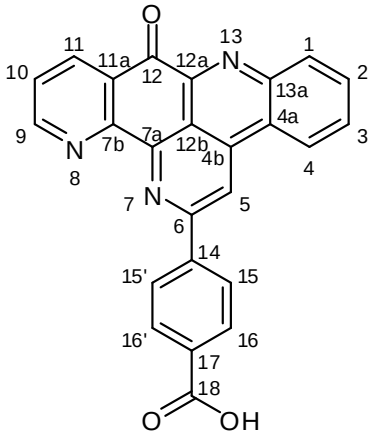


2.32

Methyl 4-formylbenzoate and **2.9** were refluxed in acetic acid, in the presence of ammonium chloride and under a nitrogen atmosphere for 1 hr to produce **2.32**. The acidic reaction mixture was diluted with water and neutralised with concentrated ammonia solution. The aqueous mixture was extracted with large volumes of dichloromethane and the combined dichloromethane was in turn washed with water. Compound purification was undertaken by preparing a slurry with a small volume of dichloromethane, filtering the slurry and washing the solid with several portions of dichloromethane. This procedure made use of and highlighted the acutely insoluble nature of the compound. No molecular ion was detected in the mass spectrum and due to the insoluble nature of **2.32** only the ^{1}H NMR spectrum was recorded. The ^{1}H NMR spectrum was consistent with the expected product, with the absence of a triplet

at δ_H 7.53 associated with the lack of H-17 as observed for **2.13**, the addition of a methoxy signal at δ_H 3.99 and the change of a 2H triplet at δ_H 7.59 ppm to a 2H doublet at δ_H 8.28 ppm corresponding to H-16,16'. These differences are as expected for a methyl ester group at position 17.

In an effort to prepare a presumably water soluble carboxylate salt, the hydrolysis of **2.32** was initially attempted by two methodologies. The first attempt was made by refluxing **2.32** with sodium hydroxide in a mixture of water and methanol. The reaction mixture turned brown over time and eventually orange on cooling. The resulting ^{1}H NMR spectra, taken in both and D_2O and d_6-DMSO, were extremely broad and whilst they indicated the presence of aromatic functionality the molecular structure of the product could not be determined. The molecular ion was not detected under FAB mass spectroscopy. The second approach attempted was that of refluxing **2.32** in concentrated HCl. The resultant material gave ^{1}H NMR data identical to that generated from base hydrolysis, namely four broad aromatic signals.

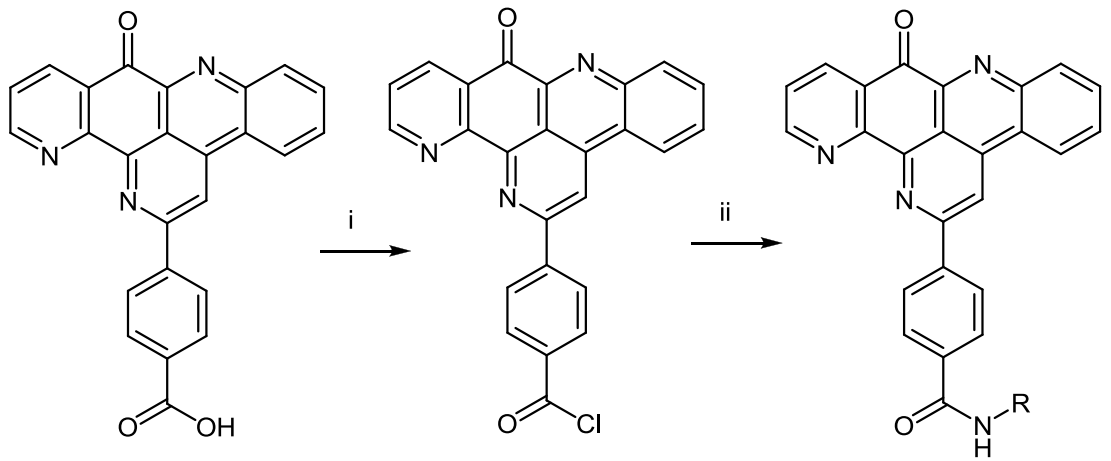


2.33

Noting that some literature examples utilized methanolic potassium hydroxide for hydrolysis of methyl esters, base hydrolysis was reexamined.[89] Overnight reflux of **2.32** suspended in 1M methanolic potassium hydroxide was followed by the removal of methanol in vacuo. The resulting solid was treated with 1M methanolic hydrochloric acid and washed with water giving the orange free acid **2.33**. While the mass spectrum of the product did not contain an identifiable molecular ion, the ^{1}H NMR spectrum was resolved and contained signals of a number, integral and multiplicity expected for the structure. Most importantly the spectrum did not show a signal expected for a methyl ester functional group, indicating the successful

hydrolysis of the methyl ester. Since the spectrum was taken in d_6-DMSO it was not sufficiently comparable with the previously collected spectra, both taken in *d*-chloroform, of methyl ester precursor **2.32** or phenyl analogue **2.13.** Due to the lack of product solubility it was not practical to gain a ^{13}C NMR spectrum but 2D COSY and HSQC experiments were completed. The ^{1}H NMR data was assigned with COSY correlations and by comparison with derivatives prepared from **2.33**.

The acid **2.33** provided the opportunity to introduce varied functionality through reaction with the carboxylic acid group. Amide formation could introduce moieties that were greatly more soluble than the parent molecules. To achieve this, **2.33** was refluxed in thionyl chloride to give acid chloride **2.34** (Scheme 2.11) which was then used to couple directly with the appropriate amines: The amines were chosen for structural simplicity and the presence of a solubilising functional group.



2.33 **2.34** **2.35** R = $CH_2CH_2CH_2N(CH_3)_2$.HCl
2.36 R = $CH_2CH_2N(CH_3)_2$.HCl

Scheme 2.11 General scheme for the formation of ascididemin derived amides.
Reagents and conditions: i) thionyl chloride, ii) RNH_2, dichloromethane.

The reaction procedure developed for this amide coupling involved the addition of significant excess of reacting amine, in this case 3-(dimethylamino)propylamine, in anhydrous dichloromethane to a dry sample of freshly prepared acid chloride (**2.34**) and stirring for 1 hour to ensure complete reaction. The excess amine neutralised any unwanted hydrochloric acid generated during reaction and the crude product, yielded from a basic workup, afforded ^{1}H NMR data in keeping with the target compound **2.35**.

Initial attempts at purification of **2.35** as the free base via silica gel flash chromatography failed to yield pure products. An alternative approach involved changing the workup to acidic conditions. The crude reaction mixture was extracted with aqueous hydrochloric acid, the combined aqueous layers then were washed with dichloromethane and dried in vacuo. The resulting red salt was purified by C18 flash chromatography and Sephadex® LH20 size exclusion chromatography.

The ^{1}H and ^{13}C NMR data observed for **2.35** showed chemical environments, integrals and multiplicities that were in agreement with the expected structure. High-resolution mass spectrometry gave the molecule formula for **2.35** ($C_{30}H_{26}N_5O_2$). Full assignment of the NMR spectra was undertaken utilising COSY, HSQC and HMBC 2D NMR experiments.

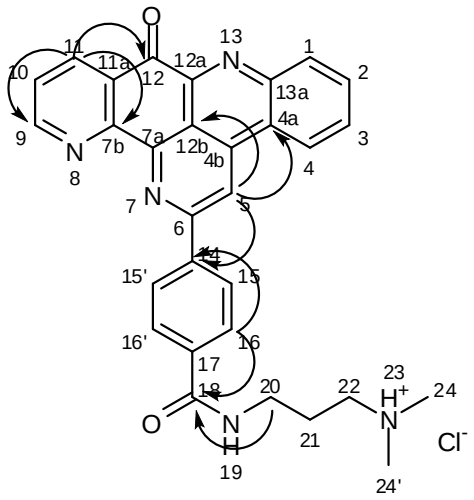


Figure 2.5 Selected HMBC correlations observed for **2.35**

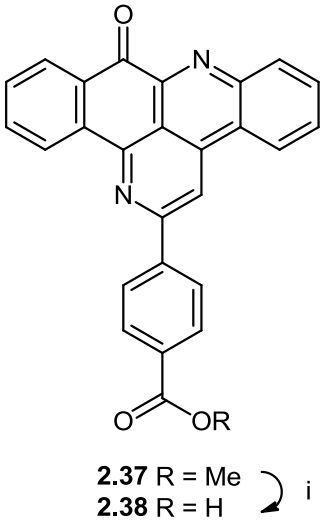
Figure 2.5 summarises HMBC NMR correlations observed for **2.35** which readily enabled the unambiguous assignment of all ^{1}H and ^{13}C NMR resonaces. The only ^{1}H NMR aromatic singlet present in the spectrum of **2.35** can be unambiguously assigned as H-5 (δ_H 9.48, 1H, s) and from this assignment HMBC, HSQC and COSY correlations were used in an alternating fashion to assign the ^{1}H and ^{13}C NMR signals for positions 1-6, 12b, 13a, and 14-24. The remaining unassigned carbonyl signal (δ_C 181.0) was then assigned as C-12 and in turn HMBC correlations to C-12 along with HSQC and COSY correlations allowed for the assignment of the ^{1}H and ^{13}C NMR signals for positions 7b, 9 ,10, 11 and 11a. Finally the remaining two uncorrelated quaternary carbons, C-7a (δ_C 148.9) and C-12a (δ_C 146.1), were assigned by

comparison with various previously made examples of the ascididemin moiety and later confirmed by comparison with the data of the 8-deaza counterpart **2.40**.

With the successful preparation of **2.35**, homologous amide **2.36** was prepared in similar fashion by addition of *N*,*N*-dimethylethylenediamine, dissolved in dichloromethane, with freshly prepared acid chloride **2.34** (Scheme 2.11). Acidic workup followed by chromatography on reverse phase C_{18} and size exclusion LH20 columns gave the desired product which was fully characterised by mass spectrometry and NMR spectroscopy. The NMR data observed for **2.36** was assigned by the same process used for **2.35** with the aromatic portions of the molecule possessing near identical signals to those of **2.35.** The chemical shifts observed for the dimethyl amino group (δ_H 2.88, δ_C 42.4) of **2.36** were similar to those observed for **2.35** (δ_H 2.80, δ_C 42.1) while the alkyl chain for **2.36** gave two methylene quartets (δ_H 3.72, δ_C 34.6; δ_H 3.34, δ_C 56.0) consistent with an ethyl chain as opposed to the three methylene propyl chain present in **2.35**. These observations provided further confidence in both sets of NMR spectral assignments.

Amides **2.35** and **2.36** exhibited excellent water solubility as well as solubility in mixes of water and methanol, however, when dissolved in pure methanol and allowed to stand both aggregated to form a plate-like precipitate.

Having prepared water soluble examples of 6-substituted ascididemin analogues, attention turned to preparing their 8-deaza counterparts.



Scheme 2.12 Hydrolysis of methyl ester **2.37** to acid **2.38**.
Reagents and conditions: i) KOH, CH_3OH

Tetracycle **2.9** was annulated with methyl 4-formylbenzoate and ammonium chloride via heating at reflux for 1 hr under a nitrogen atmosphere to afford **2.37**. A neutralising workup gave a yellow solid which in turn was purified via washing with small portions of dichloromethane and water. The product's molecular ion was not detected in FABMS, however, given a significant number of scans, the ^{1}H NMR spectrum was resolved. This ^{1}H NMR spectrum was in good agreement with the expected structure, possessing the expected variations from the phenyl substituted molecule **2.29** including the presence of a singlet (δ_H 4.00, 3H), associated with the methoxy group.

Following the methodology developed for the hydrolysis of **2.32**, the methyl ester **2.37** was refluxed overnight in methanolic potassium hydroxide (Scheme 2.12). After workup the resultant solid was characterised in the usual manner. While a molecular ion was not detected by mass spectrometry, the target acid **2.38** was structurally characterised by NMR spectroscopy (Figure 2.6). The distinctive ^{1}H NMR signal for H-5 (δ_H 9.27, 1H, s) along with the ^{13}C NMR signal for C-12 (δ_C 181.0) enabled full NMR assignment of the data (Figure 2.6).

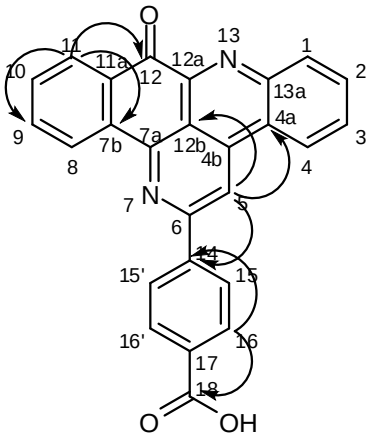
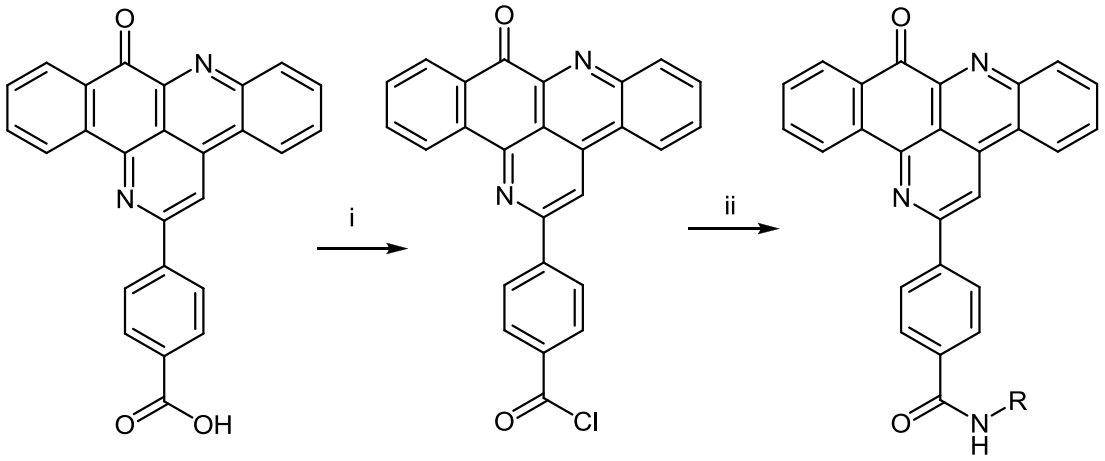


Figure 2.6 Selected HMBC correlations observed for acid **2.38**.

Acid chloride **2.39** was prepared by heating the acid **2.38** at reflux in thionyl chloride under a nitrogen atmosphere. Excess thionyl chloride was removed in vacuo and the resultant acid chloride was used as is in the following step (Scheme 2.13).



2.38 **2.39**

2.40 R = $CH_2CH_2CH_2N(CH_3)_2$
2.41 R = $CH_2CH_2N(CH_3)_2$
2.42 R = CH_2CH_2-Morpholinyl

Scheme 2.13 Synthesis of amido 8-deaza-ascididemin analogues.
Reagents and conditions: i) thionyl chloride, ii) RNH_2, dichloromethane

In a similar fashion to the preparation of amides **2.35** and **2.36**, reaction of acid chloride **2.39** with an excess of 3-dimethylamino propyl amine in dichloromethane yielded, after workup and chromatography, the desired amide **2.40**. Acidic workup was followed by C18 reverse phase and LH20 column chromatography. Full assignment of the 1D NMR data was completed via the use of COSY, HMBC and HSQC NMR experiments. While the NMR spectroscopic assignments of 8-deaza-6-phenyl-ascididemin were determined in a different solvent, the signals for the core

pentacycle (H1-11, C1-13a) of **2.40** were readily identified with similar chemical shifts, matching integrals and matching proton splitting patterns. The remaining aromatic signals for a *para* disubstituted phenyl substituent were in turn readily assigned (δ_H 8.61, δ_C 127.2, H C-15; δ_H 8.11, δ_C 127.7, H C-16; δ_C 140.1, C-14; δ_C 135.1, C-17). The remainder of the molecule was in turn assigned starting from the dimethylamino group (δ_H 2.78, 6H, d, J = 4.9 Hz, δ_C 41.9) using COSY correlations to step from proton signal to proton signal up the alkyl chain and in turn assign associated carbon signals with HSQC correlations.

Amide **2.41** was prepared in a similar procedure where *N*,*N*-dimethylethylenediamine, as a solution in dichloromethane, was slowly added to freshly prepared acid chloride **2.39**. The resultant solution was stirred under a nitrogen atmosphere for 1 hr. Acidic workup provided the hydrochloride salt which was purified by sequential C18 reverse phase chromatography and LH20 size exclusion chromatography. The FAB mass spectrum for **2.41** contained the molecular ion *m/*z 473. Further confirmation of the formation of the desired product was given by ^{1}H and ^{13}C NMR spectra which contained resonances of the expected number and chemical shifts. Furthermore the NMR data for **2.41** had signals for the aromatic portion of the molecule (H 1-16, C 1-17) that unambiguously corresponded to the same positions as those in **2.40**. The alkyl section of the molecule possessed a protonated dimethylamino group (δ_H 2.88, 6H, d, J = 4.9 Hz, δ_C 42.3; δ_H 10.65), two methylene groups (δ_H 3.74, 2H, q, J = 5.8 Hz, δ_C 34.6; δ_H 3.35, 2H, q, J = 5.7 Hz, δ_C 56.0), and an amide group (δ_H 9.16, δ_C 166.1). The relative positioning of these functional groups was determined by COSY and HMBC experiments.

The preparation of the morpholino-amide **2.42** was conducted in an analogous fashion. 2-(4-Morpholino)ethylamine, as a solution in dichloromethane, was added to freshly prepared acid chloride **2.39** and the reaction was stirred under nitrogen for 1 hr. Following acidic workup, the repeated application of C18 reverse phase chromatography produced clean (>99% by HPLC) material as the hydrochloride salt. Full assignment of the NMR data of **2.42** was achieved by analysis of 2D NMR experiments (COSY, HSQC, HMBC, ROESY). As with **2.41**, the core moiety (H 1-16, C 1-17) was easily identified when the NMR data for **2.42** was compared to that

of **2.40**. The additional signals observed indicated the presence of an amide group (δ_H 9.20, δ_C 165.9), two coupled methylene groups (δ_H 3.81, 2H, q, J = 5.7 Hz, δ_C 33.7; δ_H 3.40, 2H, q, J = 5.5 Hz, δ_C 55.6) and a morpholine group (δ_C 63.1, 51.2; δ_H 4.01, 3.92, 3.62, 3.19) as shown in Figure 2.7.

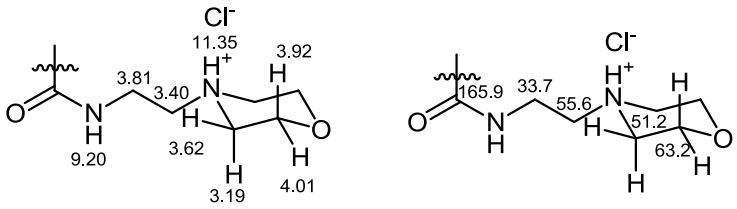


Figure 2.7 ^{1}H NMR (left) and ^{13}C NMR (right) assignments for the amide portion of **2.42**.

The proton signals observed for the morpholinyl group were assigned α or β with the aid of ROESY correlations which readily determined the stereochemical relationship of each proton in the spin system, this separation of proton signals is presumable due to slow ring inversion with respect to the NMR timescale.

Preliminary biological evaluation (discussed more fully in section 2.2.2) indicated that 8-deaza analogues (**2.11**, **2.28**, **2.29**, **2.30**, **2.31**) exhibited potent antitubercular activity but showed less cytotoxicity compared to their 8-aza (ascididemin) analogues. In addition, 6-styryl analogues were found to be more potent growth inhibitors of *Mycobacterium tuberculosis* than the corresponding 6-phenyl analogues. Thus it was decided to prepare a new series of compounds with these desirable features: 8-deaza and 6-styryl substituted containing a solubilising amido-alkyl amine functional group (Figure 2.8).

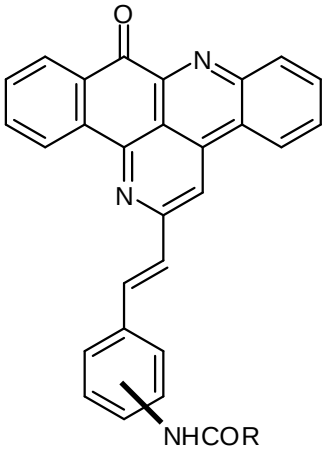
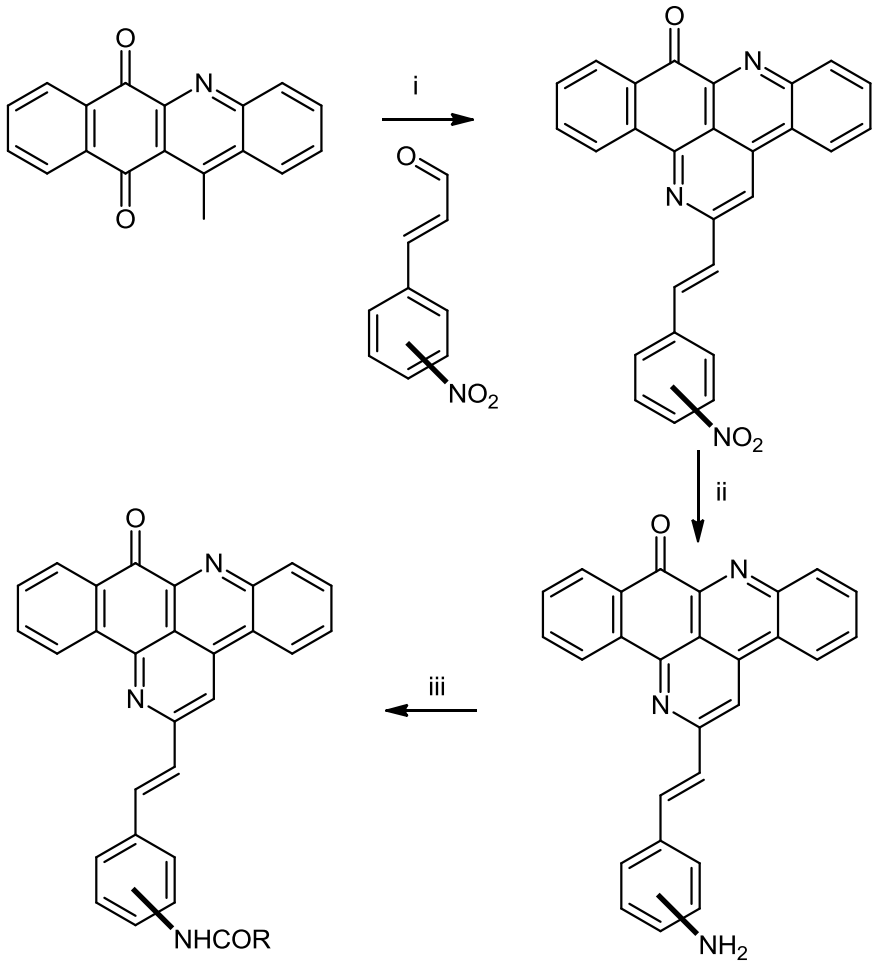


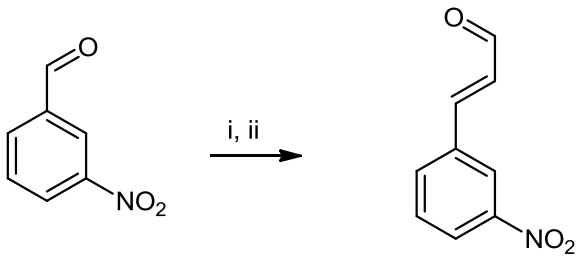
Figure 2.8 General structure of styryl amides targeted for predicted more optimal solubility, toxicity and activity.

The preparation of this class of compounds was proposed to arise from the appropriate nitro analogue being reduced to the amino analogue and in turn coupled to an appropriate acid chloride (Scheme 2.14).



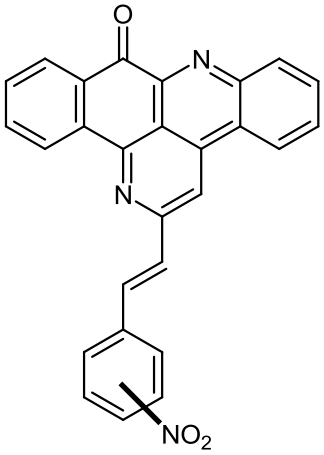
Scheme 2.14 Proposed synthesis of styryl amido analogues.
Reagents and conditions: i) NH_4Cl, AcOH, ii) reduction, iii) RCOCl

While 2- and 4-nitrocinnamaldehyde were commercially available, 3-nitrocinnamaldehyde was prepared following literature procedure (Scheme 2.15).[90] Here, methanolic potassium hydroxide was slowly added to a ice-cooled stirred suspension of 3-nitrobenzaldehyde in acetaldehyde, followed by the addition of acetic anhydride and heating to 120°C for 1 hr. The addition of concentrated hydrochloric acid to the reaction mixture and subsequent reflux gave rise to the crude product as a precipitate. Recrystallisation of this material from ethanol gave the desired product 3-nitrocinnamaldehyde with the expected NMR spectral properties which agreed with literature values.[91]



Scheme 2.15 Preparation of 3-nitrocinnamaldehyde
Reagents and conditions: i) KOH, Ac_2O, CH_3CHO, MeOH, ii) HCl

8-Deaza-6-(4-nitro-*E*-styryl)-ascididemin **2.45** was prepared following the previously established methodology. Here, ammonium chloride, 4-nitrocinnamaldehyde and tetracycle **2.9** were stirred in refluxing acetic acid under a nitrogen atmosphere. The reaction mixture was cooled, diluted with water and neutralised. The resulting brown suspension was found to take many (>10) extractions with dichloromethane to fully extract. In order to greatly reduce solvent use, the suspension was filtered through cotton wool and the solid washed with water and small portions of dichloromethane which removed the relatively soluble impurities. The cotton wool was in turn repeatedly extracted with dichloromethane overnight and the combined extracts then dried in vacuo and found to give relatively clean product in good yield (73%). The highly insoluble nature of **2.45** limited NMR studies to acquisition of only a ^{1}H NMR spectrum. The resulting spectrum contained the signals associated with the core 8-deaza-6-substituted-ascididemin moiety (H 1-4, H-5, H 8-11) as well as four additional signals: an *E* alkene (δ_H 8.24, 7.60, 1H, d, J = 15.7 Hz) and signals for a *para* disubstituted phenyl group (δ_H 8.32, 7.87, 2H, d, J = 8.8 Hz). HRFABMS gave the expected parent ion (*m/z* 430).



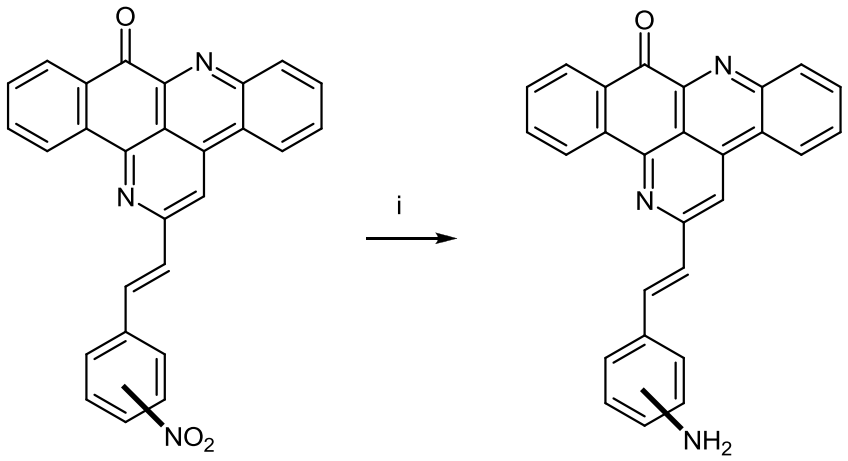
2.43 *ortho*
2.44 *meta*
2.45 *para*

Preparation of 8-deaza-6-(3-nitro-*E*-styryl)-ascididemin **2.44** was completed with a mixture of tetracycle **2.9**, 3-nitrocinnamaldehyde and ammonium chloride in acetic acid being refluxed under nitrogen for 1 hr. The product resembled **2.45** in terms of solubility and was isolated in a similar style whereby it was filtered using cotton wool, washed and finally extracted with dicloromethane. The ^{1}H NMR spectrum of **2.44** gave data in keeping with the expected structure with identifiable signals associated with the core pentacycle (H 1-4, H-5, H 8-11) matching those for **2.45** with the addition of signals for an *E* alkene (δ_H 8.26, 7.62, 1H, d, J = 15.8 Hz) and a *meta* substituted phenyl group (δ_H 8.60, s; 8.22, d, J = 9.6 Hz; 8.01; 7.92 (overlapped so multiplicity not fully observed)). The lack of solubility prevented ^{13}C NMR characterisation however the HRFAB mass spectrum showed the anticipated molecular ion at *m/z* 430.

The preparation of 8-deaza-6-(2-nitro-*E*-styryl)-ascididemin **2.43** was accomplished in a similar manner from tetracycle **2.9**, 2-nitrocinnamaldehyde and ammonium chloride. The crude reaction product was found to be much more soluble than analogues **2.44** and **2.45** and so was worked up in the standard fashion. Purification was undertaken via silica gel flash chromatography followed by washing the material with small portions of dichloromethane. FAB mass spectrometry confirmed the molecular mass while ^{1}H and ^{13}C NMR studies gave data in agreement with the expected structure. Full assignment of the NMR data was carried out with the aid of 2D NMR experiments: HMBC, HSQC and COSY. As with the other examples, H-5 (δ_H 8.40) and C-12 (δ_C 182.2) were distinct and readily identifiable and their HMBC NMR correlations allowed for the full assignment of the molecule. The assigned data was in keeping with **2.30** for the main pentacyclic moiety and the remainder of the molecule consisted of an *E* alkene (δ_H 8.63, 1H, d, J = 15.6 Hz, δ_C 130.4; δ_H 7.45, 1H, d, J = 15.6 Hz, δ_C 132.2) and an *ortho* disubstituted phenyl group (δ_H 8.08, 1H, dd, J = 8.2, 1.3 Hz, δ_C 125.0; δ_H 7.54, 1H, td, J = 7.8, 1.3 Hz, δ_C 129.1; δ_H 7.72, 1H, m, δ_C 133.3; δ_H 7.94, 1H, bd, J = 7.8 Hz, δ_C 128.5).

With the substituted nitro analogues in hand, attention turned to reduction of the nitro group to an amine. There are numerous methods used for this transformation ranging

from hydrogenation catalysed by palladium on carbon through to more traditional tin reduction methodologies. Mindful of the presence of the potentially reducible alkene functionality, initial attempts at reduction focussed on the use of a metallic tin and aqueous acid (Scheme 2.16).



2.48 2-NH_2
2.46 3-NH_2
2.47 4-NH_2

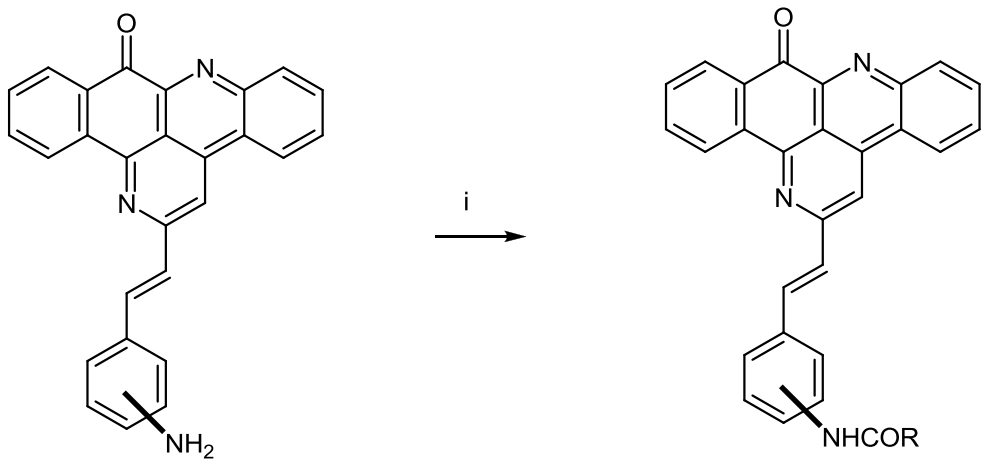
Scheme 2.16 Reduction of nitro functionality via tin metal in hydrochloric acid.
Reagents and conditions: i) Sn, conc. HCl.

The preparation of 8-deaza-6-(3-amino-*E*-styryl)-ascididemin **2.46** was accomplished by overnight reflux of a mixture of nitrostyryl **2.44** and 10 equivalents of tin powder in concentrated hydrochloric acid (Scheme 2.16). The resulting mixture was diluted with water, neutralised with sodium bicarbonate and made basic with sodium hydroxide. Extraction with dichloromethane and filtration through cotton wool removed any tin residue and gave crude reaction product which was purified by reverse phase C18 flash chromatography using MeOH/H_2O/HCl. Conversion to the free base yielded material that was too insoluble for analysis by ^{13}C NMR spectroscopy, however, the ^{1}H NMR data acquired was consistent with the expected structure. In addition to ^{1}H resonances corresponding to the pyridoacridone skeleton, signals were also observed for an *E* alkene [δ_H 8.10 (1H, d, J = 15.9 Hz), 7.42 (1H, d, J = 15.8 Hz)], a 1,3-disubstituted phenyl group (δ_H 7.25 (1H, obsc), 7.15 (1H, bd, J = 7.5 Hz), 7.05 (1H, s), 6.72 (1H, dd, J = 7.9, 1.8 Hz)) and an amino group (δ_H 3.77 (2H, br s)). The expected molecular ion (*m/z* 400.14503, calcd for $C_{27}H_{18}N_3O$ 400.14499) was observed in HRFABMS.

With the reduction of the 3-nitro analogue **2.44** proceeding smoothly the equivalent tin reduction method was applied to the 4-nitro analogue **2.45** to prepare amine **2.47**. After reverse phase chromatography the product was too insoluble for analysis by ^{13}C NMR spectroscopy but the ^{1}H NMR data was wholly consistent with the expected structure, with the changes from starting material being only significant for that of the *E* alkene group and the phenyl group with a upfield shift and the additional presence of an amino group (δ_H 3.90, br s). The mass spectrum showed the expected molecular ion ($[M+H]^+$, *m/z* 400), this in turn gave the molecular formula ($C_{27}H_{18}N_3O$, $[M+H]^+$) under high resolution MS analysis.

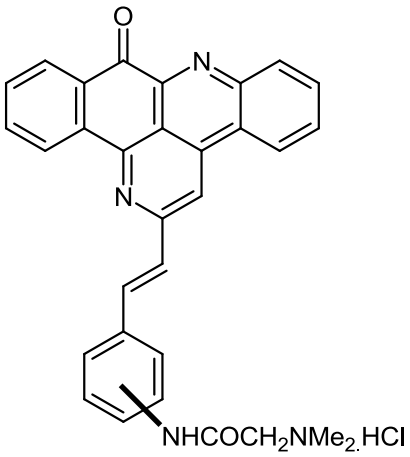
Following this methodology amine **2.48** was produced by treatment of **2.43** with tin and hydrochloric acid. Unlike the other examples of amino analogues, **2.48** was considerably more soluble and was fully characterised by ^{1}H and ^{13}C NMR spectroscopy. The full assignment of this NMR data was accomplished with the aid of 2D NMR experiments. Resonances associated with the core pentacyclic scafford were essentially identical to the starting material, but with significant upfield shifts of signals associated with the *E* styryl system [δ_H 8.16 (1H, d, *J* = 15.5 Hz, H-15), δ_C 131.4 (C-15); δ_H 7.14 (1H, d, *J* = 15.4 Hz, H-14), δ_C 127.8 (C-14)] and the addition of an amino group (δ_H 4.23, br s). Mass spectrometric data showed the molecular ion *m/z* 400, which under high resolution analysis was shown to be consistent with the molecular formula of **2.48**.

With these amino analogues in hand attention turned to their conversion to amide analogues. The first approach attempted was based on the methodology used for carboxlic acid analogues. Treating *N*,*N*-dimethylglycine hydrochloride with thionyl chloride, followed by the addition of one of the amino analogues did not, however, give the desired products most likely due to working with a very small mass of acid. Attention turned instead to the use of *N*,*N'*-dicyclohexylcarbodiimide (DCC) for amide formation (Scheme 2.17).



Scheme 2.17 Synthetic approach for amide formation.
Reagents and conditions: i) RCOOH, DCC, DMF/CH_2Cl_2

Two acids were selected for formation of amides: *N*,*N*-dimethylglycine hydrochloride, chosen for simplicity and ready availability, and 4-(2-carboxyethyl)morpholine hydrochloride, which was chosen to mimic the morpholine analogue (**2.42**) prepared earlier.



2.49 *ortho*

2.50 *meta*

2.51 *para*

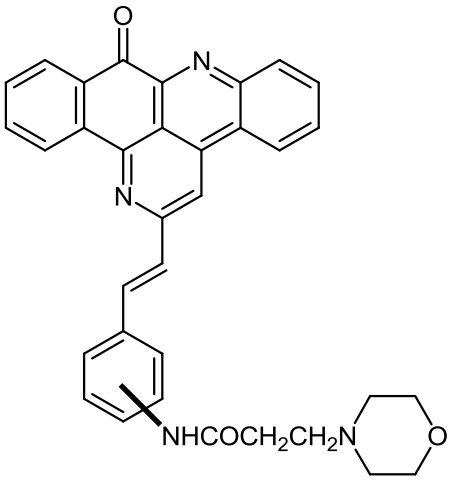
The preparation of 6-(2-*N*,*N*-dimethylglycamidyl-*E*-styryl)-8-deaza-ascdidemin **2.49** was achieved via the addition of DCC, in dichloromethane, to a solution of amine **2.48** and *N*,*N*-dimethylglycine hydrochloride in dimethylformamide-dichloromethane (0.3:1) solution. The reaction mixture was stirred under nitrogen for 66 hrs and then dried in vacuo. The solid was extracted with dilute hydrochloric acid and the aqueous solution filtered to remove any DCU. The crude product was then purified via C18 reverse phase flash chromatography with purity being monitored by reverse phase

HPLC. Characterisation of the product was primarily completed by ^{1}H and ^{13}C NMR spectroscopy, which gave data with the anticipated number of chemical environments with the appropriate chemical shifts, integrals and multiplicities. Full assignment followed via the interpretation of 2D NMR data. The signals associated with H-5 (δ_H 8.99) and C-12 (δ_C 181.1), along with that of the dimethylamino group (δ_H 2.96, δ_C 43.2), were clearly distinct in the 1D NMR spectra and 2D correlations were used to readily assign the remainder of the molecule. The data for the styryl-deaza-ascididemin moiety was well correlated with the starting amine with minor variations associated with the change in NMR solvent. The remaining signals consisted of a methylene group (δ_H 4.47, δ_C 57.9), a dimethyl group (δ_H 2.96, δ_C 43.2) and two NH groups (δ_H 11.14 and 10.32). These signals were all correlated by COSY and together make up the newly furnished dimethylglycamide HCl salt portion of **2.49**. FAB Mass spectroscopy further confirmed the products structure and the expected molecular ion of *m/z* 485 was observed.

In turn 6-(3-*N*,*N*-dimethylglycamidyl-*E*-styryl)-8-deaza-ascdidemin **2.50** was condensed from amine **2.46** and *N*,*N*-dimethylglycine hydrochloride with DCC and purified by HPLC monitored C18 flash chromatography. The products structure was confirmed by interpretation of ^{1}H and ^{13}C NMR spectra as well as the detection of the molecular ion *m/z* 485 in the FAB mass spectrum. The NMR data was fully assigned via the use of 2D NMR, and as with the analogous compound **2.49,** the signals for H-5 (δ_H 8.76), C-12 (δ_C 181.0) and the dimethylamino group (δ_H 2.95, δ_C 43.2) were used as starting points for the assignment. The NMR data for **2.50** was very similar to that of **2.49** with expected variation in the phenyl ring resonances due to the differing substitution pattern (from *ortho* to *meta*).

The 4-amido analogue **2.51** was prepared in a similar process by DCC coupling of the amine **2.47** and *N*,*N*-dimethylglycine hydrochloride and reverse phase chromatography gave sufficiently pure product as determined by HPLC. The product gave the expected molecular ion (*m/z* 485) with mass spectrometry analysis and this peak was found to be consistent with the predicted molecular formula under high resolution analysis. The NMR analysis of **2.51** gave ^{1}H and ^{13}C NMR spectra which were in keeping with the proposed molecular structure. Further NMR analysis with

2D experiments enabled the 1D data to be fully assigned. The assignment was conducted in a similar fashion to that undertaken for compounds **2.49** and **2.50** whereby H-5 (δ_H 8.05), C-12 (δ_C 180.1) and the dimethylamino group (δ_H 3.09, δ_C 44.8) were assigned from the 1D spectra and their 2D correlations enabled the assignment of the remainder of the molecule. The NMR data was in line with the data for compounds **2.49** and **2.50** with the variation arising as expected for *para* substitution on the phenyl ring.

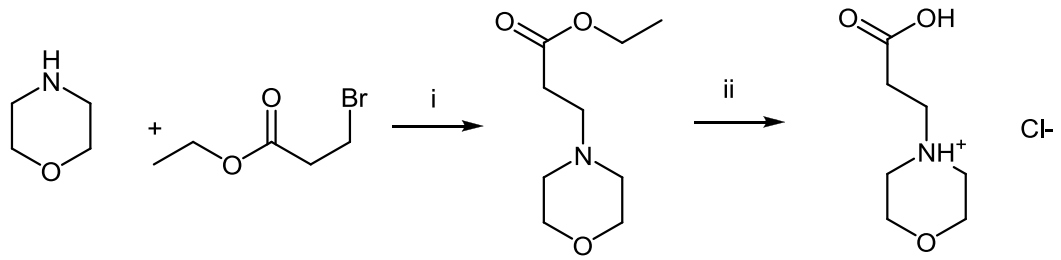


2.52 *para*

2.53 *meta*

2.54 *ortho*

Morpholine side-chain target compounds **2.52**, **2.53** and **2.54** first required the preparation of 4-(2-carboxyethyl)morpholine hydrochloride, conducted following literature procedure (Scheme 2.18).[92] Here, alkylation of morpholine with ethyl-3-bromo-proprionate yielded ester **2.55**, which upon acid catalysed hydrolysis, afforded the required carboxylic acid **2.56** in 45% yield.



2.55 **2.56**

Scheme 2.18 Preparation of 4-(2-carboxyethyl)morpholine hydrochloride.
Reagents and conditions: i) ether, ii) HCl (15%), 100 ºC.

Following the methodology developed for the glycyl analogues, the morpholine substituted *para* amide **2.52** was prepared by the reaction of amine **2.47** and 4-(2-carboxyethyl)morpholine hydrochloride **2.56** using DCC and purified by reverse phase chromatography with HPLC monitoring. The compounds FAB mass spectrum showed the expected molecular ion *m/z* 541, which was found to be consistent with the anticipated molecular formula when further analysed by under high resolution mass spectroscopy ($C_{34}H_{29}N_4O_3$). Both ^{1}H and ^{13}C NMR spectra showed the appropriate signals with intensities, integrals and multiplicities for the expected structure. The NMR signals for the aromatic portion of the molecule were readily assigned in the same manner as previous examples (**2.49**, **2.50** and **2.51**) and essentially matched those found for **2.51**. The alkyl section of the molecule was assigned by the comparison of the equivalent signals from the previous morpholino analogue **2.42** and cross checked against the 2D correlations observed.

Similarly, the preparation of *meta* amide **2.53** followed with the DCC coupling of amine **2.46** and 4-(2-carboxyethyl)morpholine hydrochloride **2.56** in dichloromethane and dimethylformamide, and purification of the product by reverse phase chromatography. The FAB mass spectrometric data showed the desired molecular ion *m/z* 541 and the 1D NMR signals were observed to have the expected chemical shifts, multiplicities and integrals. The spectroscopic peaks were fully assigned by 2D NMR experiments, using the same approach used for prior related analogues, with the assigments for the morpholino group confirmed by comparison with assignments for **2.52** and **2.42**.

Finally the *ortho* amide **2.54** was prepared following this established methodology from *ortho* amine **2.48** and 4-(2-carboxyethyl)morpholine hydrochloride **2.56**. FAB mass spectrometry/high resolution mass spectrometry detected the molecular ion (*m/z* 541) and established the molecular formula ($C_{34}H_{29}N_4O_3$) for the positive ion. The products ^{1}H and ^{13}C NMR spectra were as expected and these signals were assigned by 2D NMR in the usual fashion. The morpholino signal assignments were confirmed by comparison with the previously made morpholino analogues (**2.53**, **2.52** and **2.42**).

2.2.2 Biology

Presented in this section is data acquired by collaborators through biological evaluation of compounds supplied by the author of the thesis. The evaluation of compounds against *Mycobacterium tuberculosis* $H_{37}Rv$ was performed by Drs Helena Boshoff and Clif Barry at the National Institute of Allergy and Infectious Diseases, NIH, USA. The testing of compounds against *Escherichia coli*, *Mycobacterium smegmatis* and HL-60 assays was performed by Dr Ronan O'Toole, School of Biological Sciences, Victoria University of Wellington. Evaluation of P388 activities was performed under a fee-for-service agreement by Ms Gill Ellis, Department of Chemistry, The University of Canterbury.

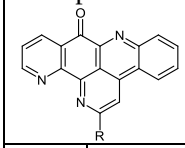
Mycobacterium smegmatis and *M. tuberculosis* are related bacteria, being of the same genus, whereas *Escherichia coli* is relatively unrelated. Comparing the activities of these three species gives a measure of general antibacterial activity as well as antimycobacterial activity and more specifically inhibition of growth activity against *M. tuberculosis*. The P-388 and HL-60 assays give a measure of the cytotoxicity of the tested compounds towards murine leukemia and human lymphocytic cell lines. By comparing cytotoxicity and antibacterial activity a measure of the compounds selectivity can be determined. The results are presented in Tables 2.1, 2.2 and 2.3. The selectivity index (SI) shown is equated to IC_{50} (cytotoxicity)/MIC (antibacterial) with values greater than 100 usually being of significant interest.

2.2.2.1 Antitubercular activity

Analysis of the 8-aza analogues antitubercular activity (Table 2.1) shows that the presence of a substituent at the 6-position moderately reduces the observed activity compared to **2.5**. For small rigidly bound substituents such as phenyl (**2.13**) or styryl (**2.14**) (between 1.5 and 5-fold reduction) and more significantly in the case where a chain is further introduced (~ 20-fold for compounds **2.35** and **2.36**). Compounds **2.35** and **2.36** are also more polar than the other examples and this may partially explain the activity change, however amine **2.25** and its hydrochloride salt **2.26** have identical activity against *Mycobacterium tuberculosis* (but not other species) and it is therefore

thought that the presence of an alkane chain in **2.35** and **2.36** partially reduces activity.

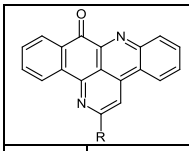
Table 2.1 Biological data for compounds **2.5**, **2.12**-**2.14**, **2.25**, **2.26**, **2.35**, **2.36**.

Compound 		*MTb*[a]	*M.smeg*[b]	*E.coli*[c]	P-388[d]	HL-60[e]	SI *Mtb HL60*[f]	SI *Mtb P388*[g]
	R							
2.5	H	0.086	9.8	14.4	0.4	0.05	0.56	4.7
2.12	CH_3	0.33	15.7	206.3	1.4	0.1	0.36	4.2
2.13	Ph	0.54	nd[h]	2.7	1.4	0.6	1.19	2.6
2.14	Styryl	0.13	nd	9.2	2.8	1.8	14.00	21.5
2.25	*p*(Me_2N)Styryl	0.46	nd	162.5	> 15[i]	0.5	1.08	>31.7
2.26	*p*(Me_2N)Styryl .HCl	0.42	0.2	5.1	2.5	0.2	0.46	6.0
2.36	*p*($Me_2N(CH_2)_2NHCO$) Ph.HCl	1.53	nd	5.8	1.8	0.4	0.27	1.2
2.35	*p*($Me_2N(CH_2)_3NHCO$) Ph.HCl	1.5	nd	2.0	2.3	0.9	0.60	1.5

[a] *Mycobacterium tuberculosis* H_{37}Rv grown in GAST medium, MIC (μM); [b] *Mycobacterium smegmatis*, IC_{50} (μM); [c] *Escherichia coli*, IC_{50} (μM); [d] P388 murine leukemia cell line, IC_{50} (μM); [e] HL60 human leukemia cell line, IC_{50} (μM); [f] selectivity index for MTb against HL60; [g] selectivity index for Mtb against P388; [h] nd, not determined; [i] values not reliable due to low solublility.

When evaluating the antitubercular activity of the 8-deaza series (Tables 2.2 and 2.3) one can note that rigid relatively non-polar aromatic substituents at the 6-position gave minimal change in activity (as opposed to the aza-series) and the styrylated compound **2.30** was slightly more active than 8-deaza-ascididemin (**2.11**). The methylated example **2.28** had a 4-fold drop in activity compared to **2.11** and each of the tethered examples (**2.41**-**2.42**, **2.49**-**2.54**) had even greater reduction in activity. The primary amino compounds **2.46**-**2.48** were also reduced in activity when compared to **2.11**. From this it would seem that the addition of more polar groups and the presence of alkyl chains lead to unfavourable antitubercular activity. However overall these compounds were still highly active against tuberculosis. It is also noteworthy that amido compounds **2.49**-**2.54** have minimal variation in antitubercular activity and this indicates that varying substitution positions on the styryl ring along with the change from a dimethyl amino termination group to a morpholino termination group has little effect on the antitubercular activity.

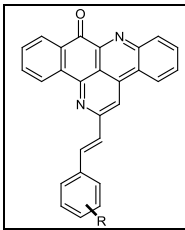
Table 2.2 Biological data for compounds **2.11**, **2.28**-**2.31**, **2.40**-**2.42**.

		*MTb*[a]	*M.smeg*[b]	*E.coli*[c]	P-388[d]	HL-60[e]	SI *Mtb HL60*[f]	SI *Mtb P388*[g]
2.11	H	0.043	0.5	nd	1.6	4.2	98.60	37.2
2.28	CH_3	0.16	nd	nd	6.1	2.2	13.88	38.1
2.29	Ph	0.068	nd	nd	16	186.5	2743.24	235.3
2.30	Styryl	0.032	nd	17.4	insol	0.5	14.28	nd
2.31	*p*(Me_2N)Styryl	0.057	58.9	181.5	> 15[i]	16.5	289.12	>256.5
2.41	*p*($Me_2N(CH_2)_2NHCO$)Ph.HCl	0.19	nd	nd	1.8	nd	nd	9.5
2.40	*p*($Me_2N(CH_2)_3NHCO$)Ph.HCl	0.37	nd	15.5	1.1	0.7	1.84	3.0
2.42	*p*($O(CH_2)_2N(CH_2)_2NHCO$)Ph.HCl	2.84	127.4	224.8	> 45	26.0	9.14	>16.0

[a] *Mycobacterium tuberculosis* H_{37}Rv grown in GAST medium, MIC (μM); [b] *Mycobacterium smegmatis*, IC_{50} (μM); [c] *Escherichia coli*, IC_{50} (μM); [d] P388 murine leukemia cell line, IC_{50} (μM); [e] HL60 human leukemia cell line, IC_{50} (μM); [f] selectivity index for MTb against HL60; [g] selectivity index for Mtb against P388; [h] nd, not determined; [i] values not reliable due to low solublility.

When comparing the 8-deaza compounds (Table 2.2, **2.11**, **2.28**-**31**, **2.40**, **2.41**) with their aza equivalents (Table 2.1, **2.5**, **2.12**-**14**, **2.25**, **2.35**, **2.36**) it can be noted that the 8-deaza series are all more active than their aza conterparts, with the difference in activity ranging from 2-fold to 8-fold. This appears to counter the claim that iron-chelation[77] plays a pivotal role in the antitubercular activity of ascididemin, as the 8-deaza series lacks a site suitable to chelate iron.

Table 2.3 Biological data for compounds **2.46**-**2.54**.

		*MTb*[a]	*M.smeg*[b]	*E.coli*[c]	P-388[d]	HL-60[e]	SI *Mtb HL60*[f]	SI *Mtb P388*[g]
2.48	*o*-NH_2	0.12	nd	219.6	>63	0.1	0.88	>522.2
2.46	*m*-NH_2	0.98	nd	nd	>63	93.1	94.99	>64.0
2.47	*p*-NH_2	0.48	142.6	205.4	insol	72.8	151.71	nd
2.49	*o*Me_2NCH_2 NHCO.HCl	0.36	7.2	nd	39	2.3	6.33	108.1
2.50	*m*Me_2NCH_2 NHCO.HCl	0.36	4.7	nd	14	4.1	11.31	37.9
2.51	*p*Me_2NCH_2 NHCO.HCl	0.18	12.2	nd	2.1	8.4	46.72	11.8
2.54	*o*$O(CH_2)_2N(CH_2)_2$ NHCO.HCl	0.33	7.4	nd	17	3.4	10.42	50.1
2.53	*m*$O(CH_2)_2N(CH_2)_2$ NHCO.HCl	0.68	5.7	nd	>43	4.1	5.99	>63.7
2.52	*p*$O(CH_2)_2N(CH_2)_2$ NHCO.HCl	0.33	12.0	nd	6.0	41.2	124.85	18.2

[a] *Mycobacterium tuberculosis* H_{37}Rv grown in GAST medium, MIC (μM); [b] *Mycobacterium smegmatis*, IC_{50} (μM); [c] *Escherichia coli*, IC_{50} (μM); [d] P388 murine leukemia cell line, IC_{50} (μM); [e] HL60 human leukemia cell line, IC_{50} (μM); [f] selectivity index for MTb against HL60; [g] selectivity index for Mtb against P388; [h] nd, not determined; [i] values not reliable due to low solublility.

Overall, the most active compound was found to be the 8-deaza-styryl derivative **2.30** which was found to have an MIC of 0.032 μM, very high activity when compared to rifampicin (MIC 0.24 μM). Also of note are the water soluble compounds **2.41** and **2.51** with MIC's 0.19 and 0.18 μM, respectively, showing activity on par with rifampicin.

2.2.2.2 General antimicrobial activity

To gain a measure of the antimicrobial selectivity of this class of compounds, a selection of analogues were evaluated against the Gram-positive bacterium *Mycobacterium smegmatis* and the Gram-negative baterium *Escherichia coli*. While the IC_{50} values generated by screening against these organisms are not directly comparable with those of MIC determined against *M. tuberculosis*, it is reasonable to expect that the MIC for these organisms would require a higher molar concentration than the IC_{50}s measured. With this in mind, general comparisons between the activity against the two microbes and *M. tuberculosis* can be made.

From the data (Tables 2.1, 2.2, 2.3) all examples have a higher IC_{50} value against *Escherichia coli* when compared to the MIC for *Mycobacterium tuberculosis*, and in some cases, as high as a 1000-fold difference. This difference in activity as well as considerable variation in activity towards *E. coli* suggests that the activity of this class of compound towards *M. tuberculosis* is independent of their general antimicrobial activity.

The IC_{50} data of ascididemin analogues against *Mycobacterium smegmatis* showed a similar trend with these compounds significantly less active for this organism compared to *M. tuberculosis*. Interestingly there appears to be no correlation between the two activities (*M. tuberculosis* and *M. smegmatis*) with more activity against *M. tuberculosis* often coinciding with less activity towards *M. smegmatis*. Since *M. tuberculosis* and *M. smegmatis* are related bacteria this observation shows that this class of ascididemins exhibit antibacterial activity highly selective towards the more pathogenic mycobacterial species, *M. tuberculosis*.

2.2.2.3 Cytotoxicity

Cytotoxic effects of the ascididemin analogues were evaluated against two cell lines, the P388 murine leukemia cell line and the HL60 human leukemia cell line. The data acquired showed little consistency with some compounds exhibiting more toxicity in the P388 assay and others being significantly more toxic in the HL60 assay. From this, one must conclude that there is a differing mechanism, be they barriers to cell penetration or mechanism of action, relating to the toxicity of each compound in each assay. From the data only broad trends could be surmised.

Cytotoxicity in the P388 assay varied wildly with the different ascididemin analogues with activity ranging from an IC_{50} of 0.4 μM (ascididemin, **2.5**) to >63 μM (**2.46**, **2.48**). Overall trends were not very distinct but all bar one 8-aza analogue (**2.25**, which is more likely to be inactive due to insolubility) exhibited IC_{50} values of 0.4-2.8 μM while the equivalent 8-deaza analogues (**2.11**, **2.28**-**2.31**, **2.40**, **2.41**) had IC_{50} values ranging from 1.1-16 μM. Thus, there appears to be reduced cytotoxicity with

the 8-deaza series. Within the pairs of analogues all but one pair (**2.35**, **2.40**) had the same or less cytotoxicity for the 8-deaza molecule compared to the aza counterpart.

Of interest is the comparison of the two sets of *ortho*, *meta* and *para* amido styryl deaza analogues (**2.49**-**52**). In both sample sets the *para* substituted derivatives were significantly more cytotoxic than the *ortho* and *meta* substituted derivatives. Notably, the toxicity of *para*-glycyl amido (**2.51**) is very similar to that of the *para* amido compounds **2.36**, **2.35**, **2.41** and **2.40** and the position of substitution may play a significant role in P388 toxicity.

As with the P388 assay, there was significant variation in cytotoxicity towards the HL60 cell line with IC_{50} values ranging from 0.05 μM to 187 μM. The 8-aza compounds again showed high toxicity in this screen with IC_{50} values of 0.05 μM to 1.8 μM in contrasted to the 8-deaza counterparts with IC_{50} values ranging from 0.5 μM to 187 μM. The majority of the 8-aza/deaza analogues were greatly less toxic for the 8-deaza examples with the most notable exception being that of the 6-styryl analogues **2.14** and **2.30** (where the 8-aza analogue is more than 3 times less toxic than the deaza counterpart).

As with the P388 data the comparison of the HL60 activity for the two sets of *ortho*, *meta* and *para* substituted amido styryl deaza analogues exhibited a trend with the *ortho* examples being the most cytotoxic and the *para* examples the least. The lack of a unified trend between activity in each of these assays suggests the need for more varied toxicity screens to gain a more accurate sense of overall activity.

2.2.2.4 Selectivity indices

From the bioactivity data selectivity indices for the compounds antitubercular activity against P388 toxicity and HL60 toxicity were calculated and are presented in Tables 2.1, 2.2 and 2.3. The indices were calculated as the ratio of the IC_{50} of the compound in the given toxicity screen to the MIC of the compound against *Mycobacterium tuberculosis*.

In general terms over both HL60 and P388 cell lines, the selectivity indices were significantly higher for members of the 8-deaza series when compared to their 8-aza counterparts. The exception to this trend was the HL60 selectivity indices observed for the styryl compounds (**2.14**, **2.30**) which were equivalent. Furthermore, all members of the styryl amido series showed good (>10) selectivity for *Mycobacterium tuberculosis* compared with P388. When these styryl analogues are examined with respect to HL60 cytotoxicity, four of these compounds still have good selectivity (>10). One should also note that the selectivity index calculation compares IC_{50} data with MIC data and can only be used to evaluate the relative selectivity of the compounds within the series. As an absolute measure, the values indicate the minimum selectivity of these compounds.

For most compounds, a high selectivity index for one cytotoxic assay was accompanied by lower selectivity in the other. The most notable exceptions, where selectivities in both cytotoxic assays are high, are **2.29** and **2.31** (deaza 6-phenyl and deaza 6-dimethylaminostyryl), but these unfortunately are not nearly as soluble as the other analogues and therefore not suited for development as an orally available therapy.

2.2.2.5 Overall summary

Overall, antitubercular activity was observed to be high for the prepared ascididemin analogues, with enhanced activity for the deaza series. This antitubercular activity has been found to be independent of other antibacterial activities. Furthermore, the cytotoxicity of the compounds has been found to be relatively inconsistent between the HL60 and P388 cell lines. Further toxicity screens may shed light on the seemly complex variations in this activity.

2.3 Conclusions

The preparation of a selection of 6-substituted ascididemin and 8-deaza-6-substited ascididemin analogues was completed. During the preparation of these compounds two viable routes for the general preparation of amide derivatives were developed. Included in this selection of derivatives were significantly more soluble analogues than that of the lead compound. Biological testing showed general retention of antitubercular activity across these analogues as well as a reduction in toxicity for most analogues with respect to the parent compound ascididemin. Some of the derivatives showed good selectively indices against *Mycobacterium tuberculosis* and, when combined with the greatly increased solubility, warrant further investigation. The most interesting examples are the 8-deaza-amido-stryl compounds (**2.49**-**2.54**) where solubility was among the highest of the examples prepared and the selectivity indices ranged from 6 (**2.49** and **2.53**) to above 100 in two cases (**2.49** and **2.54**) for the two different cytotoxicity assays tested. Further examination of the toxicity of and the synthesis of additional amido styryl analogues of these molecules is suitably justified.

2.4 Future Studies

With a novel and currently unknown mechanism of action against *M. tuberculosis,* ascididemin offers insight into potentially new target/s in the ongoing development of antitubercular agents. The molecules developed illustrated that the addition of various groups of differing length and size at the 6-position on ascididemin retains significant activity against *M. tuberculosis*. Future studies could make use of the developed amide coupling methodology in order to synthesise molecules with biologically important labels tethered to the ascididemin core structure, as this study has shown there is relatively minor reduction in activity when substituents are added to this position.

One potentially informative line of research will be that of the preparation of an affinity chromatography labelled compound. An example would be to tether biotin to the ascididemin moiety. With the use of a streptavidin-based support, such as

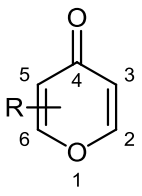
streptavidin-agarose, a modified ascididemin moiety can be tethered to this solid support. Proteins extracted from *M. tuberculosis* could be passed through this column and, after washing, the proteins bound to ascididemin could be displaced by free biotin and identified.

Labelling of ascididemin with a fluorescent tag would potentially allow for sub-cellular localisation of the active compound to be investigated. These labelled compounds could also be used to identify proteins which bind these compounds via gel electrophoresis coupled with UV-vis/fluorescence detection and hence identify protein targets.

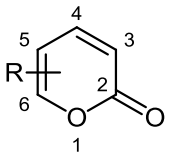
Chapter 3 2-Pyranones

3.1 Introduction

Pyranones occur naturally in two isomeric forms, as either 4-pyranones (γ-pyrones, **3.1**) or 2-pyranones (*2H*-pyran-2-ones, **3.2**). Both classes of natural products represent a large group of biologically active compounds occurring in all three kingdoms of life. While the first 4-pyranone example isolated, poppy acid, was extracted from *Papaver somniferum* in 1805, the majority of 4-pyranones have been isolated from marine organisms and have been associated with various chemical defence mechanisms.[93] 2-Pyranones have reported pharmacological activites covering a diverse spectrum including anti-inflammatory, antibacterial, antifungal, antiviral, anticancer and neuroactive activities.[94]

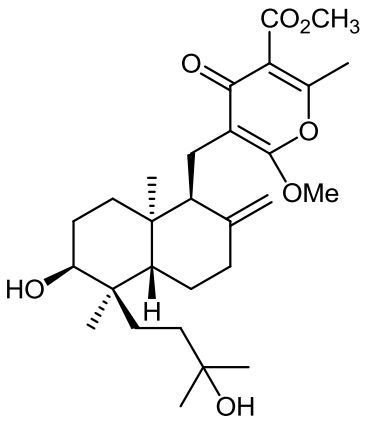


3.1

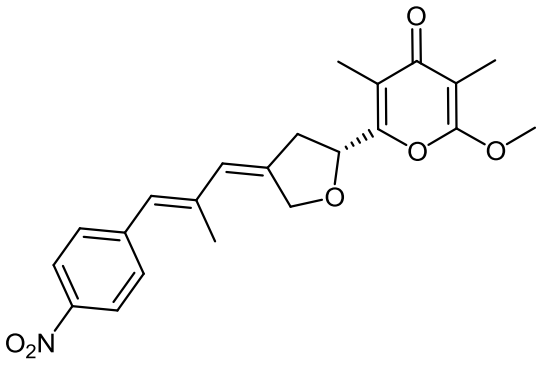


3.2

Colletotrichin A (**3.3**), isolated from a plant pathogenic fungus *Colletotrichum capsici*, is a highly toxic 4-pyranone with an LD_{50} of 16 mg kg^{-1} against rats.[95] Aureothin (**3.4**) was extracted from *Streptomyces thioluteus* and has been found to exhibit antitumour, antifungal and insecticidal activity as well as very potent activity against *Heliobacter pylori* sp.[93]

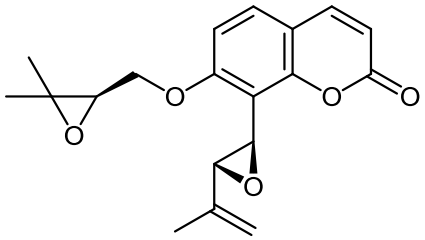


3.3

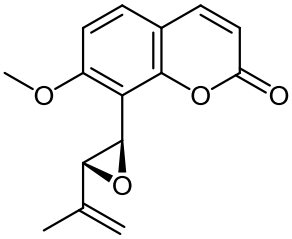


3.4

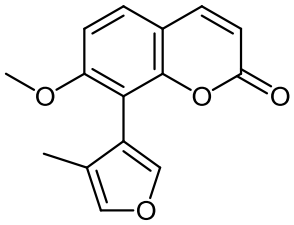
Coumarins are examples of 5,6-ring-fused 2-pyrones. Several examples isolated from the tree *Galipea panamensis* (**3.5**-**3.8**) have been reported to be as active against *Leishmania panamensis* with EC_{50} values of 9.9, 14.1 and 10.5 μg mL^{-1} for **3.5**, **3.6** and **3.7** respectively.[96]



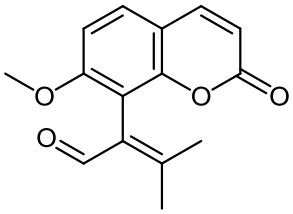
3.5



3.6

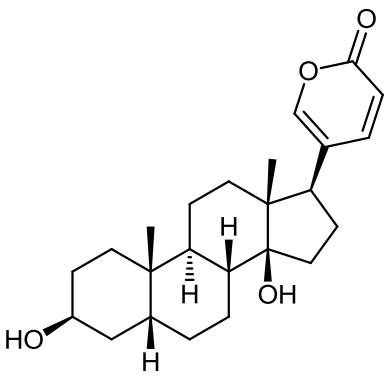


3.7



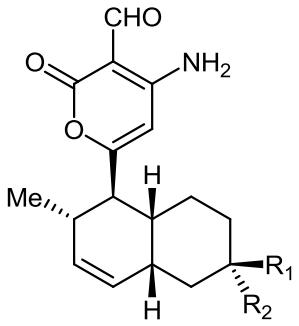
3.8

Bufalin (**3.9**) and numerous structurally diverse bufadionolides, which are characterized by the pyrone ring connected to the same core tetracyclic system as for bufalin, are sourced from animals and plants alike and exhibit immunosuppressant, pesticidal and anti-cancer properties.[94,97]



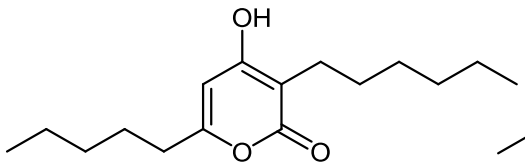
3.9

Solanopyrones E (**3.10**), F (**3.11**) and G (**3.12**), isolated from a marine fungus removed from the surface of the calcareous green alga *Halimeda monile*, possess antialgal activity.[98]

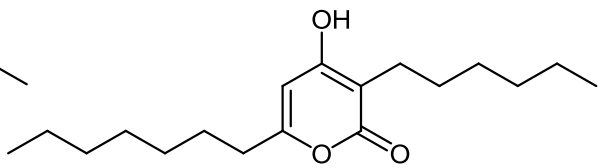


3.10 R_1 = OH, R_2 = H
3.11 R_1 = OH, R_2 = OH
3.12 R_1 = OH, R_2 = H

Pseudopyronines A (**3.13**) and B (**3.14**) are examples of alkyl substituted-4-hydroxy-2-pyranones isolated from *Pseudomonas* sp. F92S91, which in turn was isolated from a sponge in Fiji.[99] These pyrones were found to exhibit a wide range of biological activites, including growth inhibition of *Mycobacterium tuberculosis* (MIC 3.125 and 0.78-1.56 μg mL^{-1} respectively), the causative agent of tuberculosis, and antiparasitic activity against *Leishmania donovani* (IC_{50} 2.63 and 1.38 μg mL^{-1} respectively) as well as activities against *Trypanosoma brucei rhodesiense, T. cruzi* and *Plasmodium falciparum*.[47]

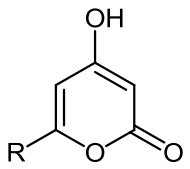


3.13

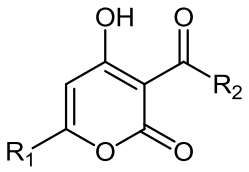


3.14

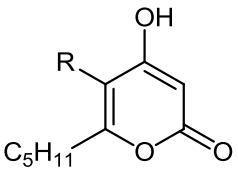
In a preliminary SAR study of **3.13** and **3.14** a library of analogues (**3.15**-**3.24**) was prepared. While no improvement on activity against *Mycobacterium tuberculosis* was found, compounds **3.18** and **3.19** showed enhanced activity against *Leishmania donovani* with IC_{50} values of 0.46 and 0.55 μg mL^{-1}, respectively.



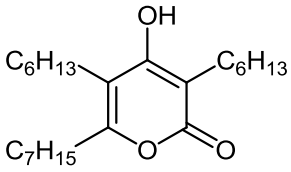
3.15 R = C_7H_{15}
3.16 R = C_5H_{11}



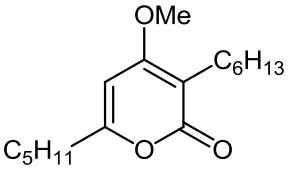
3.17 $R_1 = R_2 = C_7H_{15}$
3.18 $R_1 = R_2 = C_5H_{11}$
3.19 $R_1 = C_7H_{15}$, $R_2 = C_5H_{11}$



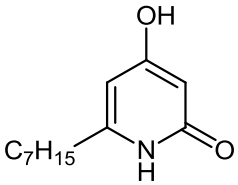
3.20 R = C_6H_{13}
3.21 R = COC_5H_{11}



3.22

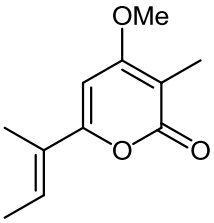


3.23

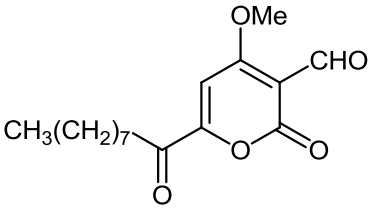


3.24

Netriapyrone (**3.25**), isolated from *Gyrostroma missouriense* and *Glicladium vermosenii*, has been reported to have antibacterial activity against *S. aureus*.[100] Phacidin (**3.26**) was isolated from the canker fungus *Potenbniamyces balsamicola* and found to be broadly antifungal.[101,102]

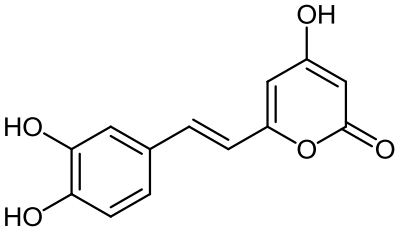


3.25

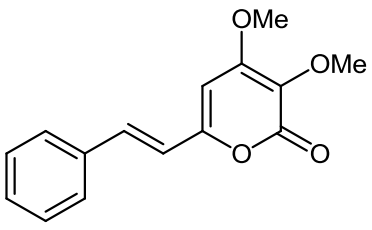


3.26

The trihydroxy styryl pyrone hispidin (**3.27**) has been isolated from a number of different sources and reported to exhibit significant antioxidant properties, selective anticancer activity,[103] and antiviral activity.[104] The structurally-related pyrone **3.28** was isolated from *Miliusa balansae*, a shrub used in traditional Chinese therapy for the treatment of gastropathy and glomerulonephropathy.[105]

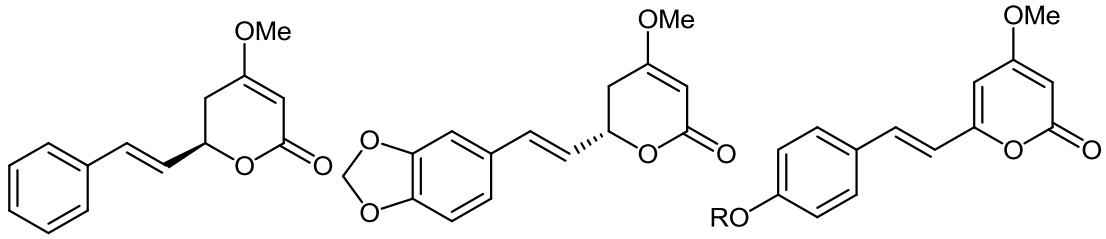


3.27



3.28

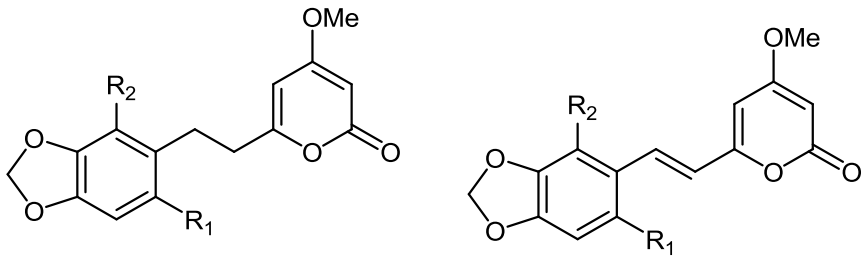
Some of the more well known pyrones are the kavalactones, having been used as a traditional intoxicant in the form of water/coconut milk extract of the roots of *Piper methysticum* for around 2000 years. The lactones confer the intoxicating properties of the extract but have also been shown to cause liver damage. It has been established that glutathione present in the traditional extracts prevent the toxic side-effects while retaining the intoxicating activity of the lactones.[106] The dominant pyrones present in kava extracts include kawain (**3.29**), methysticin (**3.30**) and yangonin (**3.31**).[106]



3.29 **3.30** **3.31** R = Me
3.32 R = H

Found in the seeds of *Alphinia blepharocalyx*, 2-pyranone **3.32** has been reported to have antiproliferative activity with an ED_{50} of 20.7 μM against murine carcinoma and 20.1 μM against human fibrosarcoma.[107]

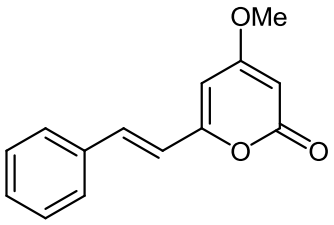
Polygala sabulosa has been used as a topical anaesthetic in folk medicine. From this species, 2-pyranones **3.33**, **3.34**, **3.35**, **3.36**, **3.37** and **3.38** have been isolated.[108,109]



3.33 R_1=R_2=H
3.34 R_1=OMe, R_2=H
3.35 R_1=R_2=OMe

3.36 R_1=R_2=H
3.37 R_1=OMe, R_2=H
3.38 R_1=R_2=OMe

Whilst screening the antimycobacterial activity of compounds extracted from *Piper sanctum*, Mata *et al.* identified 2-pyranones **3.39** and **3.33** as potential antimycobacterial leads with modest activity of MIC 32 and 4 μg mL^{-1}, respectively.[34]



3.39

3.1.1 Project aims

The aim of the project was to prepare a small library of 4-methoxy-2H-pyran-2-ones bearing various 6-styryl type substituents and their saturated analogues (Figure 3.1). With the aid of collaborators, this library would in turn be evaluated for antimycobacterial and antiparasitic activities.. The biological data obtained for these two classes of compounds would expand on the activity data for 2-pyrones **3.39** and **3.33** against *Mycobacterium tuberculosis* reported by Mata *et al*.[34] as well as build on the antiparasitic data reported by Giddens *et al*.[47] for several related 2-pyrone examples.

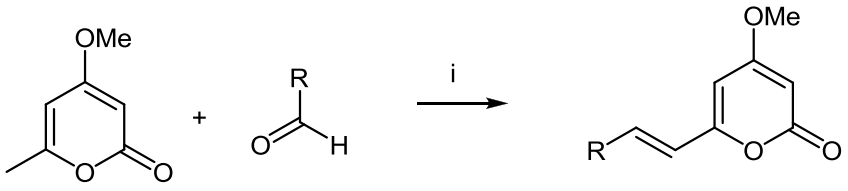
Figure 3.1 Target pyrones

3.2 Results and discussion

3.2.1 Chemistry

3.2.1.1 6-Styryl pyrones

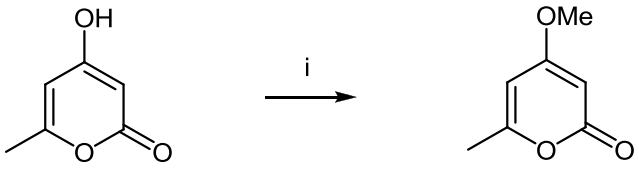
The preparation of the proposed library of pyrone analogues focused on utilizing magnesium methoxide mediated aldol condensation between 4-methoxy-6-methyl-pyran-2-one **3.40** and a variety of aryl aldehydes following an established literature procedure (Scheme 3.1).[110]



3.40

Scheme 3.1 General procedure for the preparation of 4-methoxy-6-substituted-*2H*-pyran-2-ones.
Reagents and condtions: i) $Mg(MeO)_2$, MeOH, reflux.[110]

The required methoxy pyrone **3.40** was prepared by methylation of commercially available 4-hydroxy-6-methyl-2-pyrone **3.41** (Scheme 3.2) with trimethylphosphate according to a literature procedure.[111]

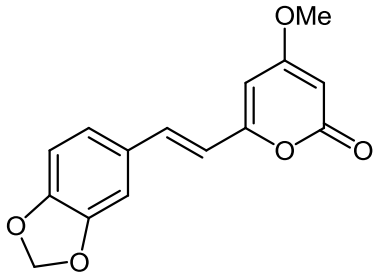


3.41 **3.40**

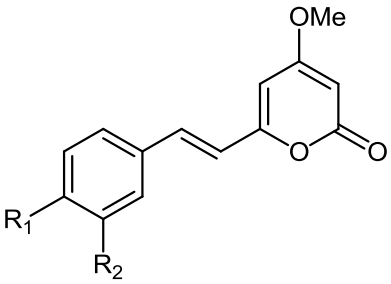
Scheme 3.2 Methylation of 4-hydroxy-6-methyl-2H-pyran-2-one
Reagents and conditions: i) trimethylphosphate, K_2CO_3, 140 °C.[111]

Water partitioning with ethyl acetate followed by silica gel flash column chromatography and recrystallisation afforded a white crystalline material **3.40**. The measured data for **3.40**, including melting point (86-87 °C), ^1H and ^{13}C NMR data and mass spectrometric data (*m/z* 141, $[M+H]^+$) were all in agreement with reported literature.[111]

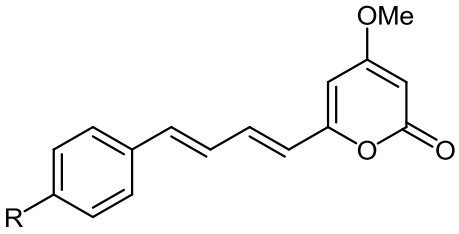
Following several literature examples, the general procedure (Scheme 3.1) was utilized for the preparation of the library of 4-methoxy-6-substituted-2H-pyran-2-ones (Figure 3.2 **3.36**, **3.39**, **3.42**-**3.55**). Here, a solution of pyrone **3.40** and the appropriate aldehyde in anhydrous methanol was added to a suspension of magnesium methoxide in anhydrous methanol and the resulting mixture heated at reflux for 6 hours. Upon cooling, the methanol was removed in vacuo. The isolation and purification of the product depended on the specific compound involved. The standard workup, used in most cases, involved the treatment of the dried crude reaction product with acetic acid and extraction with dichloromethane. The dichloromethane layer was in turn washed with water and dried in vacuo.



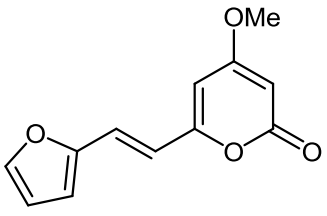
3.36



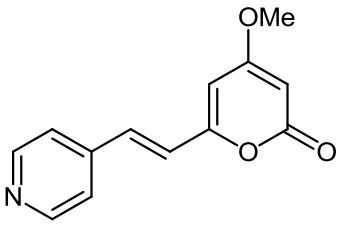
3.39 $R_1 = R_2 = H$
3.42 $R_1 = H$, $R_2 = NO_2$
3.43 $R_1 = H$, $R_2 = OPh$
3.44 $R_1 = H$, R_2 = O-Ph-*p*-*tert*-butyl
3.45 $R_1 = NO_2$, $R_2 = H$
3.46 R_1 = *tert*-butyl, $R_2 = H$
3.47 $R_1 = R_2$ =OMe



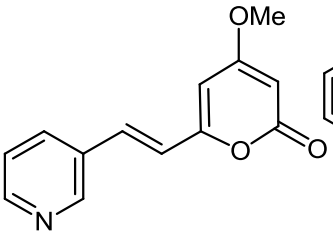
3.48 R = H
3.49 R = NMe_2



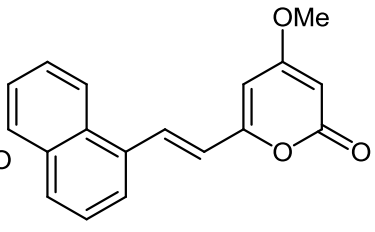
3.50



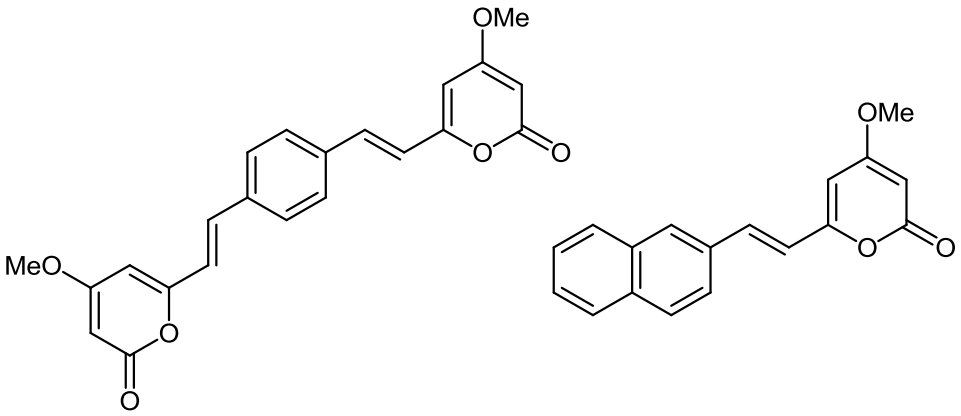
3.51



3.52



3.53



3.54 **3.55**

Figure 3.2 6-Stryl pyrones synthesised.

The 'parent' pyrone **3.39** was prepared using the general procedure reaction with benzaldehyde. The resulting oil was precipitated with small portions of diethyl ether and the resulting solid recrystallised from methanol. The ^{1}H and ^{13}C NMR data observed for the product showed distinctive signals for a 4-methoxy pyrone moiety, identified by comparison with those of the staring material. In addition signals for an *E* alkene [δ_H 7.49 (1H, obsc, H-8), 6.56 (1H, d, *J* = 16.0 Hz, H-7)] and for a mono-substituted phenyl ring were clearly identified. The assignment of these signals to the structure were made with the aid of HMBC, HSQC and COSY NMR data sets and is summarised in Figure 3.3. To make these assignments H-5 (δ_H 5.93, d, J = 2.2 Hz) was identified by the observation of an HMBC NMR correlation between the proton resonance and an alkene carbon signal (δ_C 118.6) which inturn was assigned as C-7. From these assignments HMBC, HSQC and COSY NMR correlations could be used in an alternating fashion to readily assign all NMR signals except δ_C 164.0 which did not have an observed HMBC correlation. This signal was assigned, by a process of elimination, as C-2. These assignments, along with the melting point and mass spectrum, were in full agreement with previously reported data.[112]

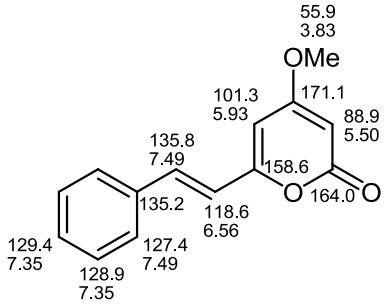


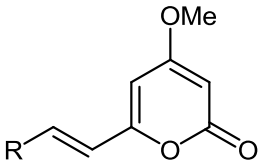
Figure 3.3 ^{1}H and ^{13}C NMR assignments for **3.39** ($CDCl_3$)

It is well established in the literature that some examples of these target pyrones can undergo photodimerisation in the solid state.[113-118] It was proposed that such photoreactions, either in the solid state or in solution may affect the yield of the targeted pyrones and so selected examples (**3.39**, **3.44**, **3.46**, **3.36**, **3.47**, **3.53**, **3.54**, **3.55**) were prepared in the presence or absence of light. In all cases, light exclusion more than doubled the observed yield of the target pyrone and in some cases increased the yield more than 5-fold (Table 3.1).

The synthesis and characterisation of target pyrones **3.39**,[119] **3.42**,[119] **3.45**,[119] **3.48**,[110] **3.51**,[110] **3.52**,[110] **3.36**,[118] **3.47**,[118] have been previously reported and in all cases the data observed in this study agreed with the previously reported values.

The ^{1}H and ^{13}C NMR data along with EI/ESI-MS spectrometric data for **3.43**, **3.44**, **3.46**, **3.49**, **3.50**, **3.53**, **3.54**, **3.55** all agreed with the relevant expected structure. The expected NMR resonances associated with the pyrone ring were present in all compounds and new resonances were observed for the various R groups consistent with the proposed structures. The full assignment of these NMR signals was accomplished in the same fashion, as discussed above, as was completed for **3.39**. (see experimental section for details).

Table 3.1 Product yields for styryl pyrones



Pyrone made	R	yield a[a]	yield b[b]
3.39	phenyl	19%	47%
3.42	3-nitrophenyl	29%	nd[c]
3.43	3-phenoxy-phenyl	23%	nd
3.44	3-(4-*tert*-butyl-phenoxy)-phenyl	4%	22%
3.45	4-nitrophenyl	5%	nd
3.46	4-*tert*-butyl-phenyl	5%	25%
3.36	benzo[*d*][1,3]dioxolyl	20%	40%
3.47	3,4-dimethoxyphenyl	ND	28%
3.48	styryl	6%	nd
3.49	4-dimethylaminostyryl[d]	5%	nd
3.50	furanyl	21%	nd
3.51	4-pyridyl	4%	nd
3.52	3-pyridyl	9%	nd
3.53	1-naphthyl	9%	40%
3.54	dipyrone	10%	27%
3.55	2-naphthyl	10%	35%

[a] product yield from standard conditions, [b] product yield by performing reaction protected from light, [c] nd, not determined, [d] was produced with modified conditions: Pyrone 3.B and *p*-dimethylaminocinnamadehyde was added in dichloromethane:methanol 2:3 and the reaction time was extended to 22 hrs.

Slow evaporation of a methanolic solution of **3.46** and diffusion of hexane into a solution of **3.47** in acetone yielded crystals of a suitable quality for X-ray analysis. It can be noted that both compounds pack in a head-to-tail fashion between layers as shown in Figures 3.4 and 3.5. The alignment of these molecules in the solid state plays a pivotal role in the types of photodimerisation reactions these molecules can undertake and this is more fully explored in Section 3.2.1.4.

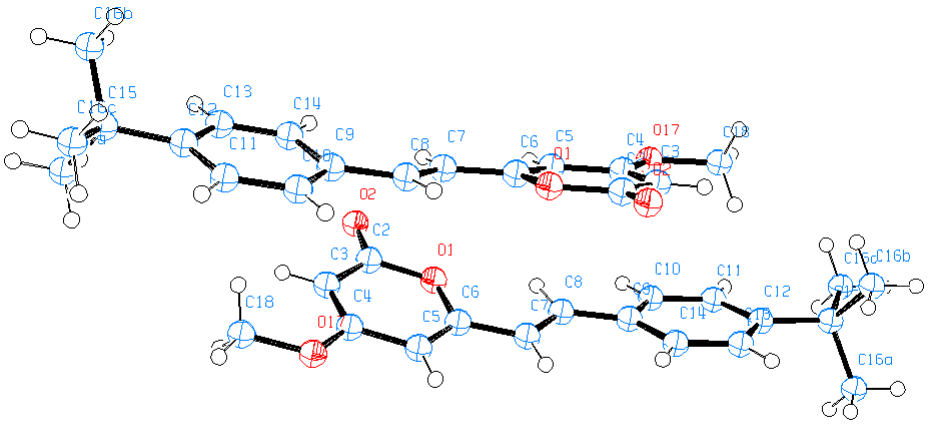


Figure 3.4 X-ray crystal structure for **3.46**.

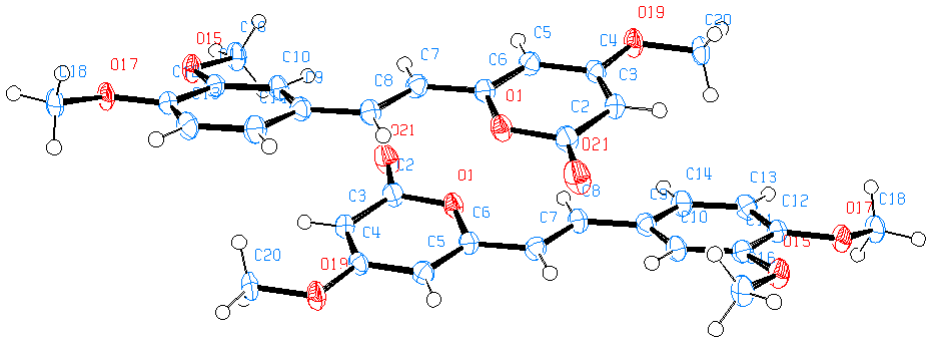
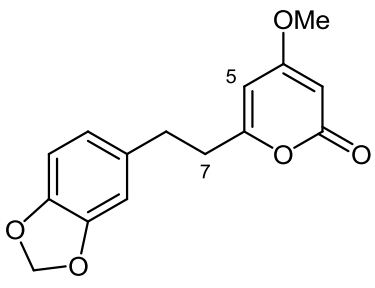


Figure 3.5 X-ray crystal structure for **3.47**.

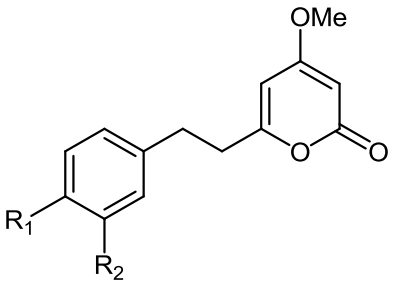
3.2.1.2 Alkyl pyrones

The Δ^7-reduced form of pyrone **3.36**, **3.33**, was identified by Mata *et al*. as an antitubercular lead compound with a MIC of 4 μg/mL towards *M. tuberculosis*.[34] Investigation of this motif and its biological activity can be accessed through hydrogenation of a selection of the styryl pyrones synthesised earlier.

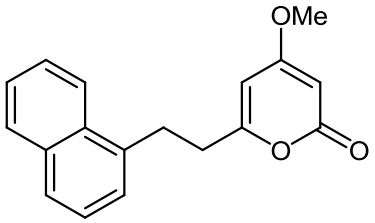


3.33

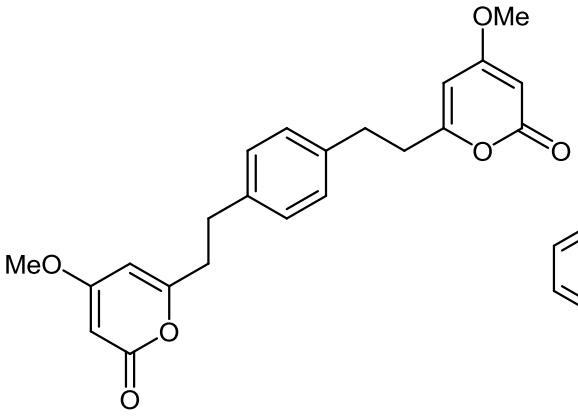
Hydrogenation of styryl pyrones has been the subject of a number of reports.[110,120,121] Most heterogeneous catalysts reduce the styryl double bond (Δ^7) first, with the Δ^5 olefin also susceptible to reduction with longer reaction times. Along with **3.33**, seven examples (**3.56**-**3.62**) were selected for synthesis, shown below:



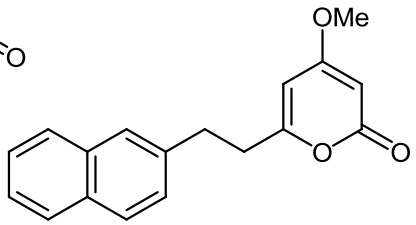
3.56 $R_1 = R_2 = H$
3.57 $R_1 = H$, R_2 = O-Ph-*p*-*tert*-butyl
3.58 R_1 = *tert*-butyl, $R_2 = H$
3.59 $R_1 = R_2$ =OMe



3.60

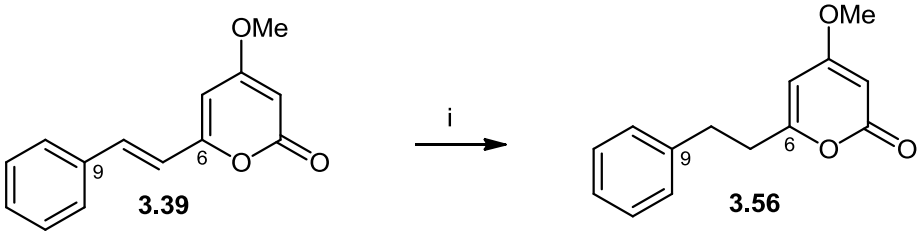


3.61



3.62

Broadly following the methodology outlined by Banerji *et al.*,[120] pyrone **3.39** was reduced to pyrone **3.56** via hydrogenation with palladium on carbon (67 % yield, Scheme 3.3). Purification of the product was achieved by recrystallisation of the crude material from water-methanol.



3.39 **3.56**

Scheme 3.3 Hydrogenation of pyrone **3.39** to give pyrone **3.56**
Reagents and conditions: i) H_2, 10% Pd/C, 1:1 MeOH:$CHCl_3$, 2 h

The ^{1}H and ^{13}C NMR spectra of the product were assigned with the aid of 2D NMR experiments. The NMR data for **3.56** (Figure 3.6) showed the expected absence of 1,2 disubstituted ethene signals, compared with **3.39**, and the introduction of signals for a 1,2 disubstituted ethane (δ_H 2.96 (2H, t, *J* = 7.2 Hz, H-8), 2.74 (2H, t, *J* = 7.2 Hz, H-7), δ_C 35.4 (C-7), 32.8 (C-8)). The NMR resonance assignments for the pyrone and phenyl rings showed some variation, compared to **3.39**, with the most significant shifts being that of position 6 (δ_C 158.6 to 164.3) and 9 (δ_C 135.2 to 139.8) as expected due to their proximity to the change in functionality. Electron impact mass spectroscopy confirmed the molecular mass (*m/z* = 230).

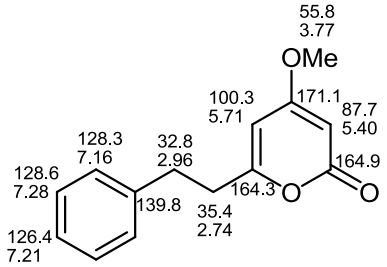


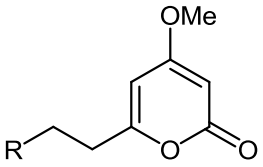
Figure 3.6 ^{1}H and ^{13}C NMR assignments for **3.56** ($CDCl_3$)

With pyrone **3.56** in hand, the same procedure was applied to synthesise the remaining seven targets. The reaction time was extended for certain examples, when preliminary reaction for 2 hours was found to give incomplete reaction, as indicated by ^{1}H NMR spectroscopy (Table 3.2). In the specific case of the synthesis of **3.61**, reaction progress could be monitored by the amount of undissolved yellow starting material-reaction completion being indicated by complete dissolution of the yellow solid.

Purification of **3.56** and **3.33** was achieved by slow recrystallisation from water:methanol, while the remaining examples were purified by flash silica gel column chromatography eluting with dichloromethane and varying percentages of methanol. All compounds were found to have the appropriate molecular ion in their respective mass spectra, which in turn gave the appropriate molecular formula under high resolution analysis. The ^{1}H and ^{13}C NMR data for these compounds were

consistent with their respective structures and were assigned with the aid of COSY, HSQC and HMBC NMR experiments (See experimental section for details).

Table 3.2 Alkyl pyrones synthesised by hydrogenation of styryl pyrones.



Hydrogenation product	R	reaction time (hrs)	yield
3.56	phenyl	2	67%
3.33	benzo[*d*][1,3]dioxolyl	2	72%
3.59	3,4-dimethoxyphenyl	2	96%
3.62	2-naphthyl	6	84%
3.60	1-naphthyl	6	75%
3.58	4-*tert*-butyl-phenyl	2	90%
3.57	3-(4-*tert*-butyl-phenoxy)-phenyl	2	92%
3.61	dipyrone	20	49%

Slow evaporation of a water-methanol solution of **3.33** gave crystals suitable for X-ray analysis. The structure (Figure 3.7) shows a kinked conformation across the ethane linkage, unlike that of the two examples of styryl X-ray crystal structures (Figures 3.3 and 3.4), both of which were essentially planar.

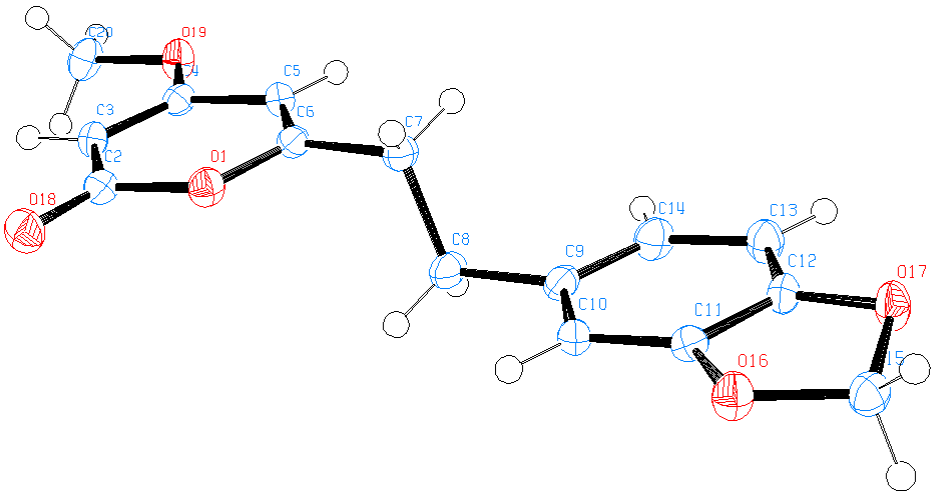


Figure 3.7 X-ray crystal structure of **3.33**.

3.2.1.3 Photodimerisation

Upon long term (14 months) storage in the dark of dried samples, a number of the pure pyrone samples were observed to have undergone reaction. ^{1}H NMR spectroscopy and ESI mass spectrometry determined that the reaction products were pyrone dimers.

4-Methoxy-6-styryl-*2H*-pyran-2-one **3.39** has been extensively studied in the literature and is known to photodimerise in the solid state to three distinct dimer forms as summarised in Figure 3.8.[113-117,122-124]

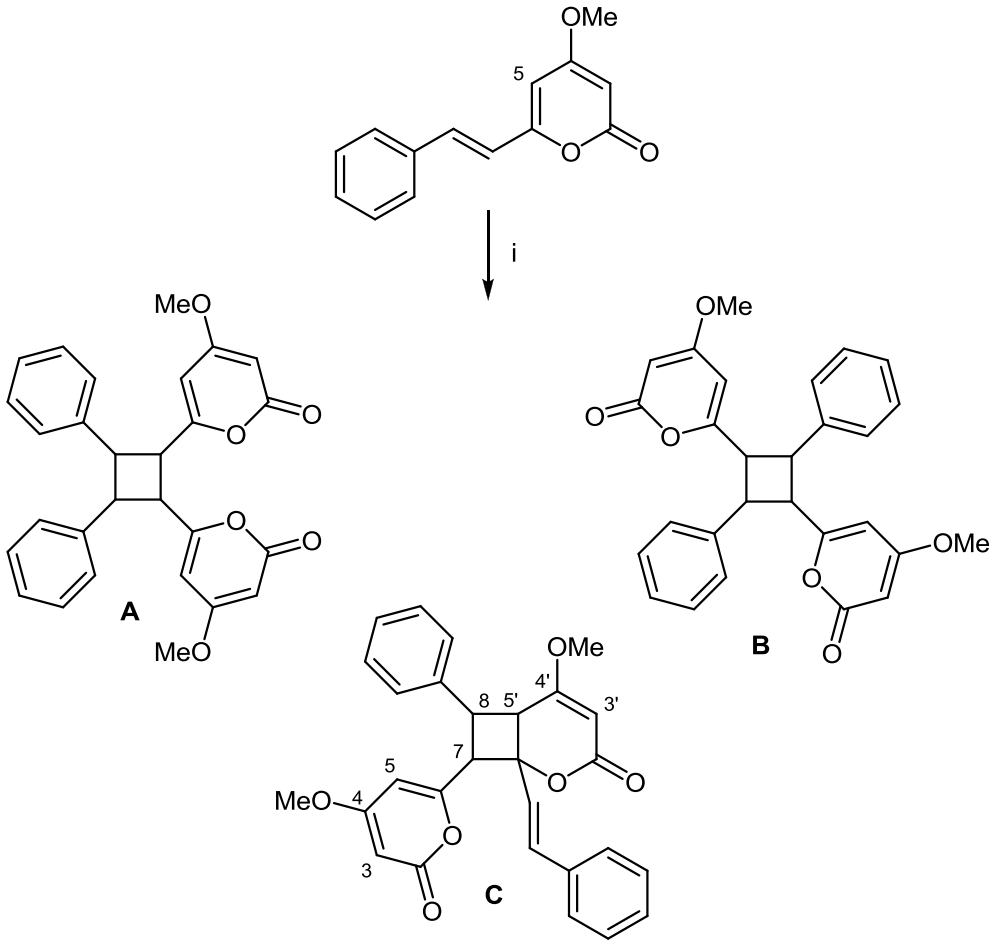


Figure 3.8 Photodimers derived from pyrone **3.39**.
Reagents and conditions: i) hv, solid state.

These dimers arise from reaction of the two photoreactive olefinic sites: the Δ^7 styryl alkene group and the Δ^5 olefin of the pyrone ring. The three established photocyclisation products **A**, **B** and **C** arise from styryl:styryl [2+2] cycloadditions in

either head-to-head (form A) or head-to-tail (form B) orientations or cycloaddition of a styryl alkene of one molecule with the pyrone Δ^5 of a second molecule (form C).

The samples that had been stored and found to contain dimers could be further evaluated in terms of the type of dimer/s present. This was achieved by identifying characteristic ^{1}H NMR resonances associated with each these respective dimer types. Type C dimers were the most distinctive with diagnostic signals for the protons on the pyrone ring systems, the cylobutane ring and the methoxy groups associated with the pyrone rings. The pyrone protons were observed as two doublets [δ_H 5.91 (1H, d, J = 2.2 Hz, H-5), 5.34 (1H, d, J = 2.1 Hz, H-3)] and a singlet [δ_H 5.28 (1H, s, H-3')], the cyclobutane ring was observed as a distinctive sequence of a triplet and two doublets [δ_H 4.35 (1H, t, J = 10.3 Hz, H-8), 4.16 (1H, d, J = 11.1 Hz, H-7), 3.59 (1H, d, J = 10.1 Hz, H-5')] and methoxy groups were observed as two well separated singlets [δ_H 3.71 (3H, s, OMe-4), 3.27 (3H, s, OMe-4')]. The section of the ^{1}H NMR spectrum of the stored sample of **3.46** with these distinctive signals is shown in Figure 3.9. Minor levels of the pyrone starting material are also discernible at δ_H 5.92, 5.47 and 3.81 ppm.

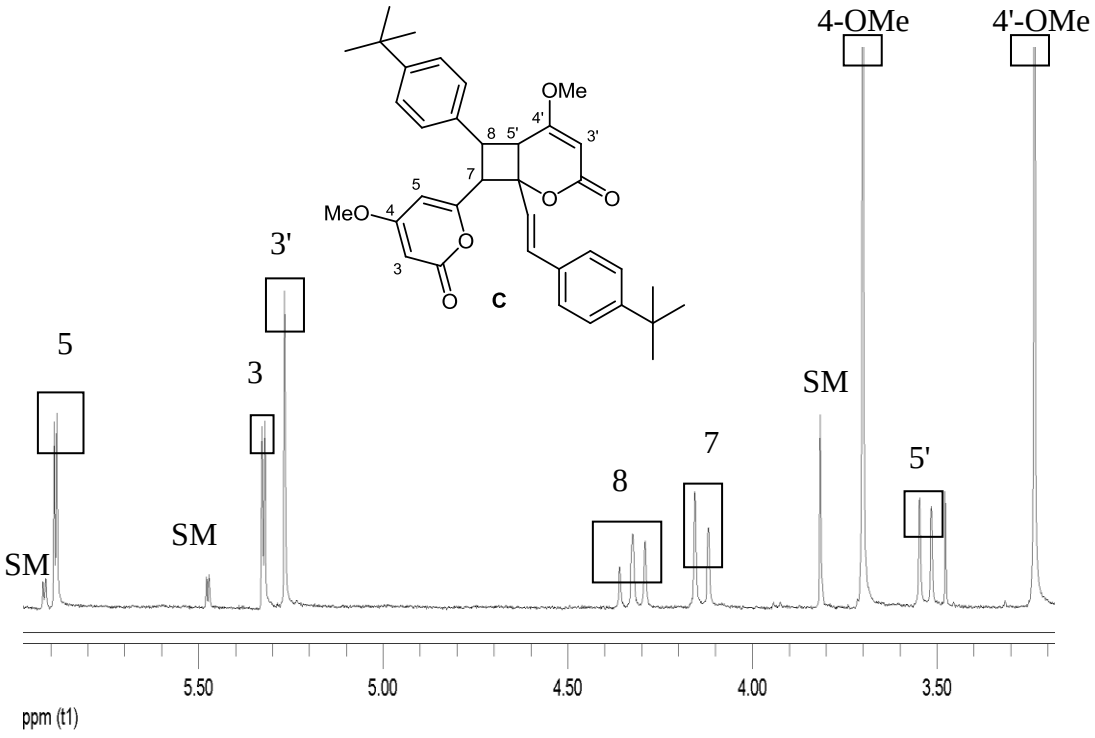


Figure 3.9 ^{1}H NMR spectrum showing diagnostic resonances typical of Type C cycloadducts. SM = starting material.

Type A and B cycloaddition products were similar to each other with most diagnostic ^{1}H NMR signals arising from the cyclobutane ring protons. Specifically, in Type A dimers, the cyclobutane signals appear as two pseudo doublets approximately 0.4 ppm apart. Such signal separation has been noted in the literature.[113,115] A representative example of the ^{1}H NMR spectrum of a Type A dimer is shown in Figure 3.10.

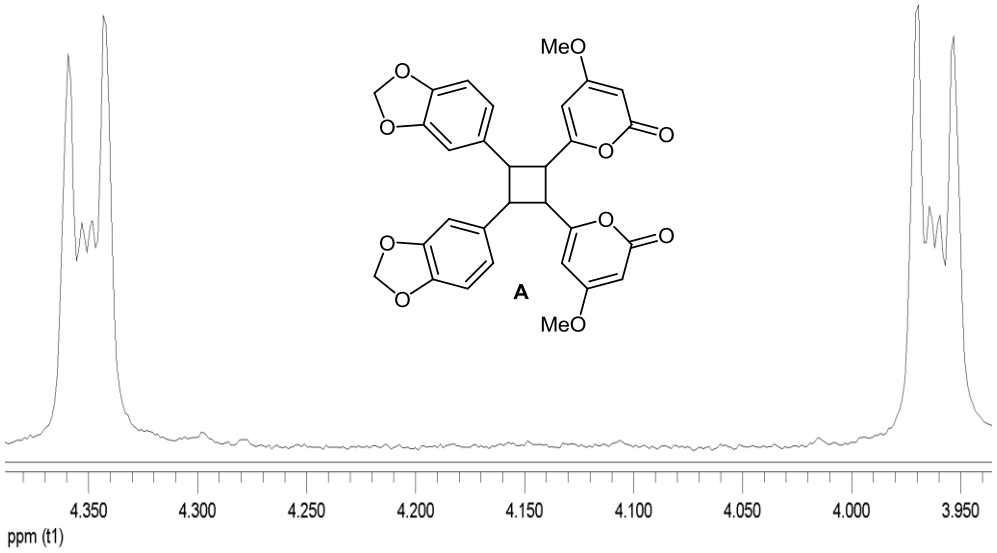


Figure 3.10 Section of the ^{1}H NMR spectrum showing diagnostic resonances typical of Type A head-to-head cycloaddition dimers.

By comparison, ^{1}H NMR signals observed for the cyclobutane protons of Type B dimers (Figure 3.11) appear as more complex multiplets resembling triplets at approximately 0.2 ppm apart, as reported in the literature examples.[118,125,126]

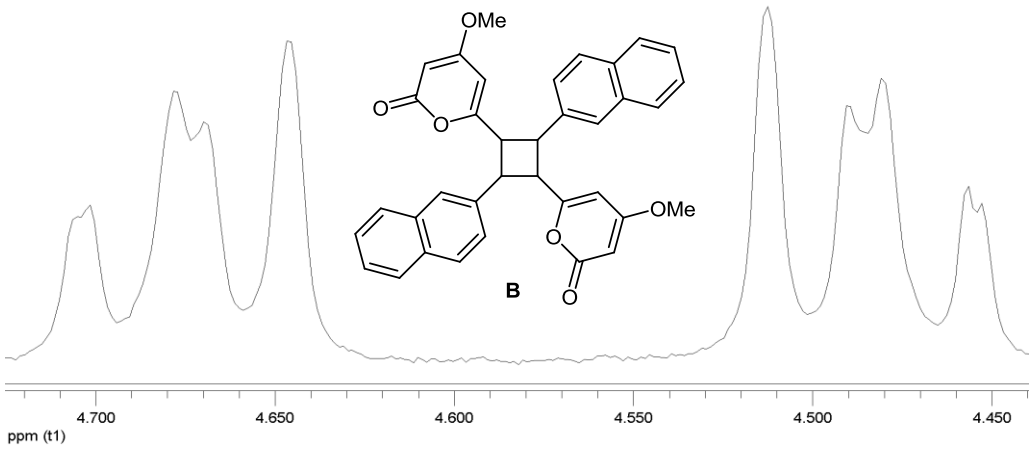
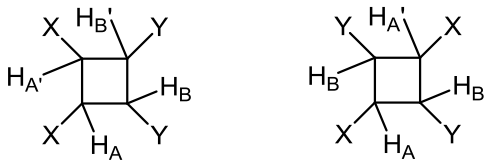


Figure 3.11 Section of the ^{1}H NMR spectrum showing diagnostic resonances typical of Type B head-to-tail cycloadduction products.

The difference between the proton NMR signals for the cyclobutane protons for dimers of type A and type B can be understood by the consideration of two parameters; the frequency separation between the peaks and the lineshape of the individual peaks. The frequency separation of the cyclobutane proton resonances can be qualitatively explained by the simplified treatment of deshielding effects of substituents on a sp^3 hybridised system. That is, the deshielding effect of a group to a given proton significantly drops with each additional sigma bond between the group and the proton. Table 3.3 summarises the number of bonds from each proton ($H_A/H_{A'}$ and $H_B/H_{B'}$) to the respective X and Y groups of dimers of types A and B (Figure 3.12). For type A dimers the groups three bonds away from both H_A's and H_B's are identical and thus the difference in chemical shift for H_A and H_B are determined by the difference in deshielding nature of groups X and Y. For type B dimers the groups three bonds away from each H_A or H_B are the opposite from the group two bonds away. Thus the proton with the more deshielding group adjacent to it has the less deshielding groups three bonds away and vice versa. This combination would be expected to exert only modest differences in shielding of $H_A/H_{A'}$ versus $H_B/H_{B'}$, resulting in small chemical shift differences between the cyclobutane proton resonances of a type B dimer. Qualitatively we therefore expect type B dimers to have a smaller difference in frequency for $H_A/H_{A'}$ and $H_B/H_{B'}$ than that of type A dimers, as observed.



A **B**

Figure 3.12 General structures for dimers of type A and type B

Table 3.3 Number of sigma bonds from cyclobutane protons to groups attached to the ring.

Dimer Type	proton	groups 2 bonds away	groups 3 bonds away	groups 4 bonds away
A	H_A, $H_{A'}$	X	X,Y	Y
A	H_B, $H_{B'}$	Y	X,Y	X
B	H_A, $H_{A'}$	X	Y,Y	X
B	H_B, $H_{B'}$	Y	X,X	Y

The difference in observed lineshape is understood by considering the spin system of the cyclobutane protons. As the H_A, $H_{A'}$ and H_B, $H_{B'}$ pairs are chemically equivalent they therefore form a AA'BB' spin system. The coupling constants of protons in a sp^3 hybridised system are highly dependant on the number of bonds between protons with ^3J coupling constants being significant (~5-10 Hz) and ^4J coupling constants being at least an order of magnitude weaker[127] (~0-0.5 Hz). Analysis of the number of bonds between protons is shown in Table 3.4.

Table 3.4 The ^3J and ^4J relationships for cyclobutane protons of dimers of type A and type B.

Dimer Type	proton	protons ^3J	protons ^4J
A	H_A	$H_{A'}$, H_B	$H_{B'}$
A	H_B	H_A, $H_{B'}$	$H_{A'}$
B	H_A	H_B, $H_{B'}$	$H_{A'}$
B	H_B	H_A, $H_{A'}$	$H_{B'}$

From this analysis two distinct parameter sets for NMR simulation are derived. For type A dimers $J_{AA'}$, $J_{BB'}$ and J_{AB} are all significant (~5-10 Hz) and $J_{AB'}$ is minor (~0-0.5 Hz). Entering these parameters into NMR simulation software,[128] using any values for $J_{AA'}$, $J_{BB'}$ and J_{AB} between 5 and 10 Hz and 0-0.5 Hz for $J_{AB'}$, spectra are generated that show peaks that appear as a wide doublet with smaller peaks between (usually two) such as observed for type A dimers. Figure 3.13 shows a representative simulation.

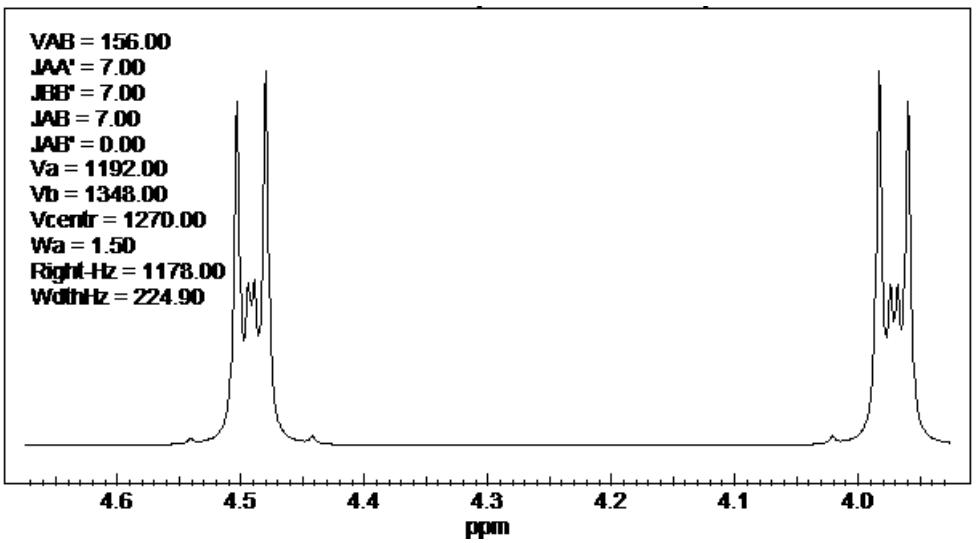


Figure 3.13 Representative simulation of type A dimer spin systems.

For type B dimers J_{AB} and $J_{AB'}$ are significant (~5-10 Hz) but $J_{AA'}$ and $J_{BB'}$ are minor (~0-0.5 Hz). Simulations of AA'BB' spin systems using these parameters generate lineshapes approximating a second ordered triplet as observed for type B dimers. Figure 3.14 shows a representative simulation which closely matches the observed spectrum shown in Figure 3.11.

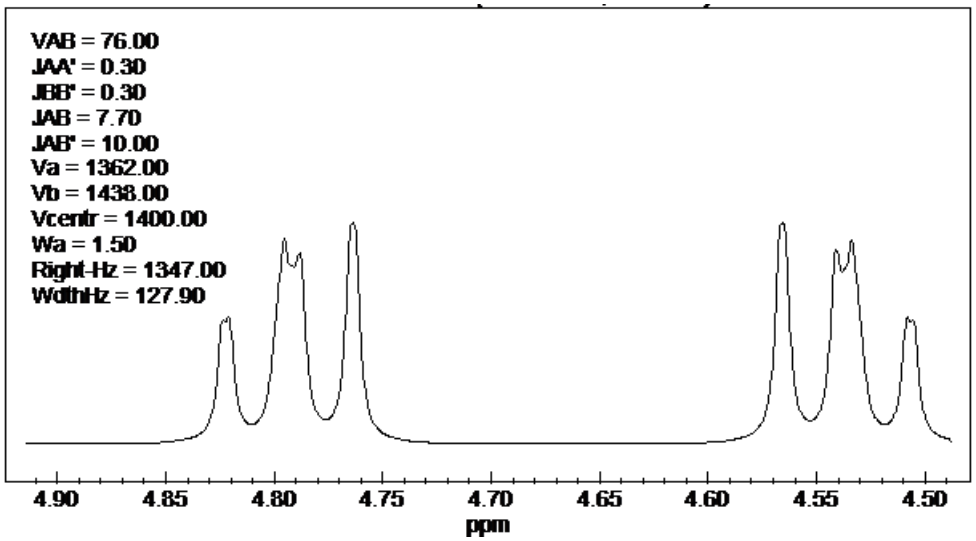


Figure 3.14 Representative simulation of type B dimer spin systems.

Using these three characteristic ^{1}H NMR fingerprints the crude reaction products were analysed and ratios of Type A, B and C dimers were determined by ^{1}H NMR signal integrations (Table 3.5). As an example, the ^{1}H NMR spectrum of the reaction product of stryl pyrone **3.39** is shown in Figure 3.15. While the more intense signals of unreacted starting material H-3 and H-5 resonances dominate, signals attributable to another product (dimer A) are clearly discernable at δ_H 5.99 and δ_H 5.36.

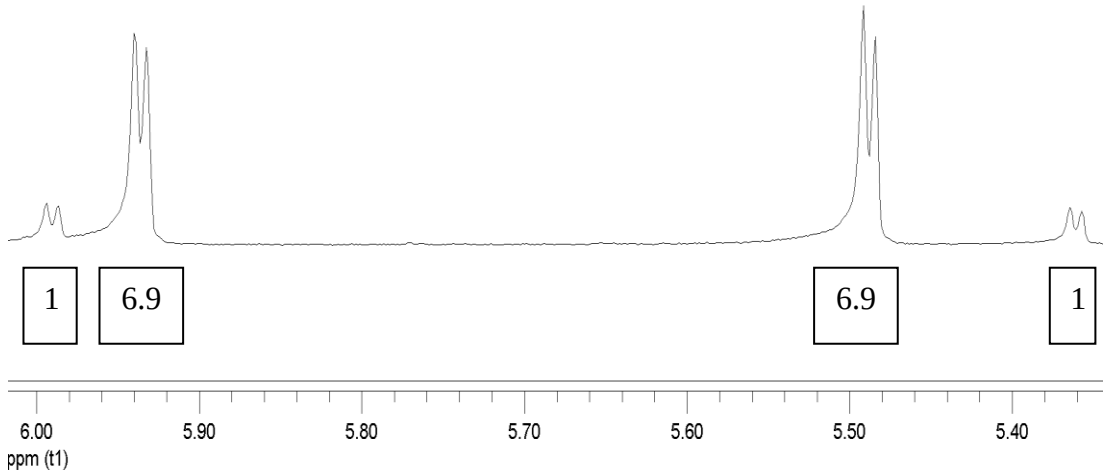
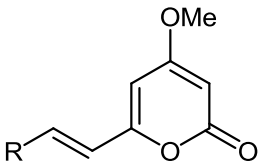


Figure 3.15 Section of ^{1}H NMR spectrum, with integrals, of stored sample of **3.39** showing ^{1}H NMR signals associated with the pyrone system for the starting material and dimer product.

Closer inspection of Table 3.5 revealed some trends among reactivity and specificity of reaction. The simplest aryl (entry 1) and pyridyl (entries 2 & 3) styryl pyrones showed formation of exclusively Type A dimerisation and it can be noted that the molecular shape these three pyrones would be expected to be very similar. *Tert*-butyl-phenoxy-phenyl and *tert*-butyl-phenyl (entries 4 & 5) styryl pyrones show very high reactivity and produce type C dimers only. Significantly, they are the only molecules present with a *tert*-butyl group and it is thought that this group dominates the crystal packing and therefore the resulting photo-dimerisation product. The 2-naphthyl pyrone (entry 6) is alone in this sample set for a preference in readily forming a type B dimer. Interestingly, this is in contrast to its 1-naphthyl isomer (entry 7) which did not react readily and exhibited only weak product NMR signals, which were too weak to allow structure elucidation. Likewise, the 3-phenoxy-phenyl pyrone (entry 8) reaction product alos exhibited weak NMR signals, preventing elucidation of the product structure. The furanyl pyrone (entry 9) yielded two dimer forms, one of Type C and either type B or A (the signals were too weak and overlapped to distinguish) and, given the relatively different shape and chemical behaviour of a furanyl group, could not be conclusively assigned. The four remaining pyrone examples (entries 10-13) showed no formation of dimers and while they do not have a particular structural feature in common they represent the four least soluble compounds stored and it is likely that these compounds were primarily in a non-crystalline form and thus not able to readily undergo solid-state photodimerisation.

Table 3.5 Ratio of storage reaction products from pure pyrones, as determined by comparison of the integrals of ^{1}H NMR signals for H-3 and H-5 of the pyrone ring.

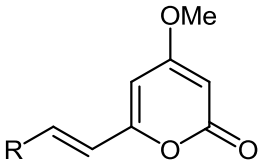


entry	molecule	R	SM[a]	form A	form B	form C
1	**3.39**	phenyl	6.9	1[b]	0	0
2	**3.52**	3-pyridyl	61[c]	1[c]	0	0
3	**3.51**	4-pyridyl	5.0	1	0	0
4	**3.44**	3-(4-*tert*-butyl-phenoxy)-phenyl	1	0	0	2.5
5	**3.46**	4-*tert*-butyl-phenyl	1	0	0	6.2
6	**3.55**	2-naphthyl	1	0	5.2	0
7	**3.53**	1-naphthyl	39[c]	1[b,c,d]	1[b,c,d]	1[c,d]
8	**3.43**	3-phenoxy-phenyl	47[c]	1[b,c,d]	1[b,c,d]	1[c,d]
9	**3.50**	furanyl	19.3	1[d]	1[d]	1.2
10	**3.36**	benzo[*d*][1,3]dioxolyl	1	0	0	0
11	**3.54**	dipyrone	1	0	0	0
12	**3.42**	3-nitrophenyl	1	0	0	0
13	**3.45**	4-nitrophenyl	1	0	0	0

[a] SM: starting material, [b] note that the signal integrated represents two protons in the dimers A or B versus one proton in the starting material or dimer C, [c] the level of signal to noise for these ^{1}H NMR spectra makes the ratio recorded only approximate, [d] the spectra was either too weak or possessed significant signal overlap such that it was not possible to distinguish between the possible structures.

Pyrones **3.49** and **3.48** possessed multiple alkene functionality and so their potential dimerisation products could not reasonably be predicted. While some dimerisation had occurred with these samples, based on the presence of additional ^{1}H NMR signals for the pyrone rings, the lack of sample size and the low yield of product meant that no structural assignment could be made. Table 3.6 gives the observed ratios of starting material to unknown dimer product for both pyrones.

Table 3.6 Ratio of dimerisation observed for samples of pyrone **3.49**, **3.48**.



molecule	R	signal ratio[a]
3.49	4-dimethylaminostyryl	10 to 1[b]
3.48	styryl	35 to 1[b]

[a] ratio of starting material to unknown dimer; [b] values are approximate due to high signal to noise.

3.2.1.4 Controlled photodimerisation

To further investigate the structures of the photochemical dimers, controlled photoreaction of various monomers was undertaken. As a preliminary trial, a stirred sample of **3.39** (10 mg) in dichloromethane solvent was subjected to irradiation using a sunlamp (300 W) from a distance of 15 cm for 3 hrs. The resulting material was dried in vacuo and ^{1}H NMR spectra indicated complete decomposition of the material to a multitude of unidentifiable products.

Following established methodology,[129] a test solid state photoreaction was attempted. A micro-crystalline sample of **3.39** (3 mg) was suspended in a small amount of water (~ 1 mL) with vigorous stirring and exposed to a sunlamp for 3 hrs. Extraction of the crude reaction product with dichloromethane and subsequent analysis by TLC and ^{1}H NMR spectroscopy indicated that the primary product was a Type A cycloadduct, with other dimers also present, as shown in Figure 3.16.

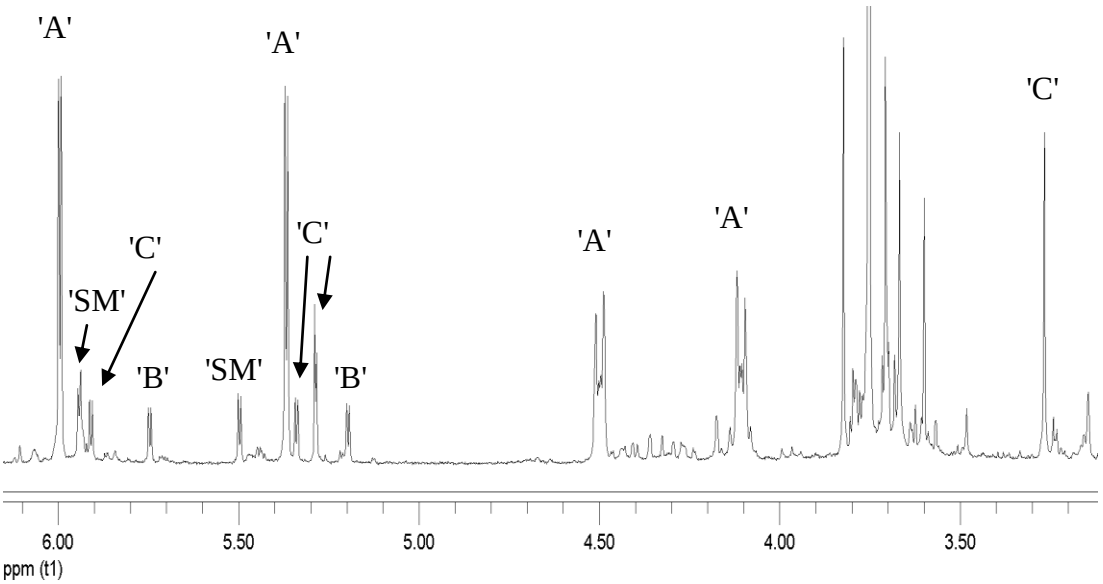
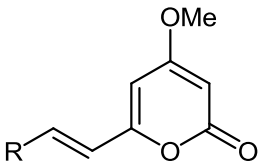


Figure 3.16 ^{1}H NMR spectrum of the crude reaction product of the photodimerisation of **3.39**.

Due to the success of this trial reaction, scale up was undertaken to enable the isolation and full characterisation of dimer products. In addition, photoreactions for **3.36**, **3.44**, **3.46**, **3.47**, **3.50**, **3.55**, **3.53**, **3.54** were attempted. Pyrones were selected due to their reactivity as a stored sample or interesting structure (e.g. 1-naphthyl versus 2-naphthyl). Pyrone **3.47** was included as it is a known natural product and its photodimers have been isolated as natural products.[118]

Samples of the selected pyrones (~ 20 mg) were suspended in water (~1 mL) with vigorous stirring and exposed to a 300 W sunlamp from a distance of 15 cm for 5 hrs. The samples were then dried in vacuo and the product ratio determined by analysis of ^{1}H NMR spectra. The procedure was repeated until either the ^{1}H NMR data showed significant loss of starting material, or a significant rise in unidentifiable complex (non-dimer) products was detected or a total of four reaction-cycles had been attempted. Since each ^{1}H NMR experiment involved dissolving and subsequent drying of the sample, the solid state packing of pyrone starting material and dimers was disrupted. Thus subsequent reactions would take place with altered solid state forms, perhaps influencing relative reaction rates of dimerisation. Hence only the first ^{1}H NMR analysis was used to determine the relative ratios of dimer products (Table 3.7).

Table 3.7 Relative ratios of the dimer products and starting material determined by ^{1}H NMR analysis of pyrone photolysis reactions.



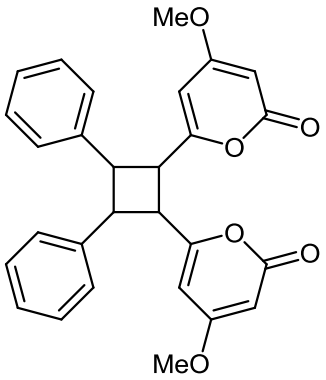
molecule	R	SM[a]	form A	form B	form C
3.39	phenyl	11.3	14.0[b]	1.1[b]	1.0
3.36	benzo[*d*][1,3]dioxolyl	3.2	1.0	0	0
3.44	3-(4-*tert*-butyl-phenoxy)-phenyl	1.0	0	0	5.1
3.46	4-*tert*-butyl-phenyl	1.0	0	0	4.8
3.47	3,4-dimethoxyphenyl	1.4	0	1.0	2.2
3.50	furanyl	3.2	0	1.0	5.1
3.55	2-naphthyl	1.0	0	19.9	0
3.53	1-naphthyl	3.6	1.0	0	0
3.54	dipyrone	1	0	0	0

[a] starting material; [b] note that the signal integrated represents two protons in the dimers A or B versus one proton in the starting material or dimer C

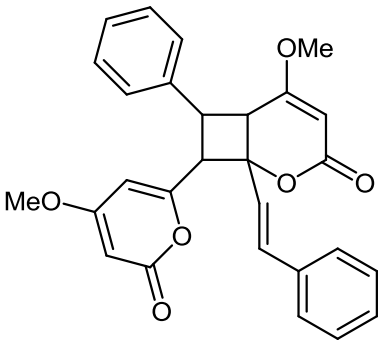
After applying the appropriate number of sunlamp exposures, as stated above, crude reaction products were purified by multiple silica flash chromatography runs eluting with a gradient solvent system starting with dichloromethane and ending with 1.0 to 3.0 % methanol/dichloromenthane depending on the samples polarity. While this solvent system worked well for the purification of the type C dimers, dimers of type A and B often smeared and consequently were isolated in lower yield than expected from the crude ^{1}H NMR data. Unfortunately the multiple sunlamp exposures led to the decomposition of the samples for **3.50** and **3.53**, which were both found to be a complex mixture of products. Chromatography of these mixtures did not yield an identifiable product. Dipyrone **3.54** showed no significant formation of dimers or decomposition after four subsequent sunlamp exposures and was not studied further.

Photoproducts were isolated from reactions of **3.39**, **3.36**, **3.44**, **3.46**, **3.47** and **3.55**.

Significant literature exists describing the photolysis of **3.39**. As stated earlier, three distinct photoproducts have been isolated by three different research groups, with Gottlieb *et al*. describing all three dimers in various publications.[113,114,117,123,124] While the ^{1}H NMR spectrum of the crude reaction product produced in this instance indicated the presence of all three dimer forms, only type A (**3.63**) and type C (**3.64**) were isolated in significant amounts.



3.63



3.64

The ^{1}H and ^{13}C NMR data observed for **3.63** were in agreement with literature data,[113,115] and the high-resolution ESI mass spectrum for **3.63** gave the appropriate ion for the expected molecular formula with *m/z* 457.1650 (calcd for $C_{28}H_{25}O_6$ 457.1646). The ^{1}H and ^{13}C NMR data were assigned with the aid of 2D NMR experiments and were found to be consistent with literature assignments. The head-to-head orientation of dimer **3.63** was assigned by lineshape analysis of the ^{1}H NMR signals observed for the cyclobutane protons, as described on page 80-83. This assignment was confirmed by analysis of ESI MS-MS fragmentation data. Figure 3.17 shows the types of mass spectometric fragmentation that can occur for both type A and type B dimers. Diagnostically, it is important to note that fragments of the 'dipyrone' (D) and 'diaryl' (E) type can only arise from a head-to-head or type A dimer. The ESI MS-MS spectrum for **3.63** exhibited ions at both *m/z* 277.0701 (calcd for $C_{14}H_{13}O_6$ 277.0707), corresponding to the 'dipyrone' type fragment D, and *m/z* 229.0853 (calcd for $C_{14}H_{13}O_3$ 229.0859) corresponding to the starting monomer type fragment F. Thus head-to-head geometry of **3.63** was established.

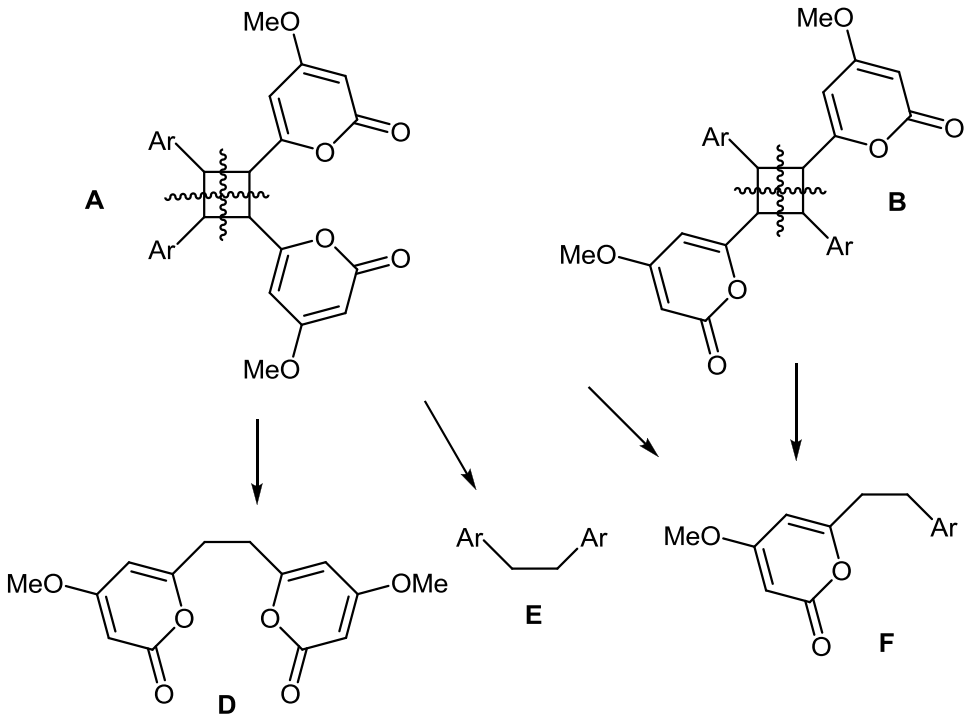


Figure 3.17 Mass fragmentation pathways for Type A and Type B cycloadducts.

[2+2] Cycloadditions are concerted and thus retain the relative configuration of the precussor alkene. Thus the *trans* relationship of the phenyl and pyrone groups is retained and so there are two potential relative configurations for **3.63**: *syn* (**3.63a**) and *anti* (**3.63b**) shown in Figure 3.18.

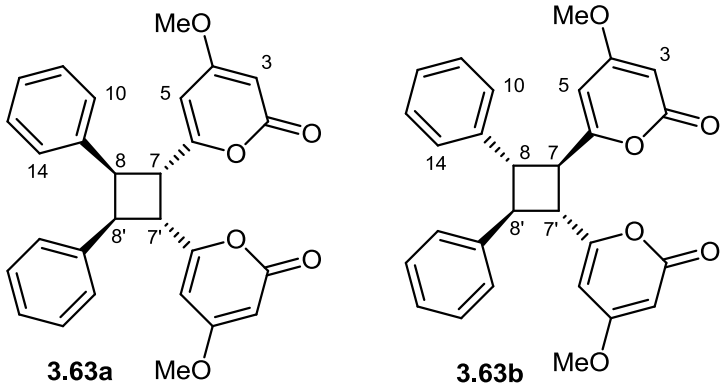
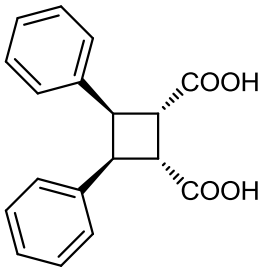


Figure 3.18 Two possible relative stereochemistries for **3.63**.

The NOESY NMR experiment for **3.63** exhibited NOE correlations from H-7 to H-8 as well as correlations between protons H-7 and H-8 and protons H-5 and H-10/H-14. While protons H-7 and H-8 are expected to correlate to protons H-5 and H-10/H-14 in either **3.63a** and **3.63b**, correlations observed between protons H-7 and H-8 would

only be expected in the *anti* case (**3.63b**) where protons H-7' and H-8 as well as H-7 and H-8' are on the same face. With the tightly packed and relatively twisted geometry that arises in cyclobutanes there is a reasonable chance that protons H-7 and H-8 would correlate in either arrangement. This is suggested by a 3.1 Å interatomic distance for protons H-7 and H-8 in **3.63a** as modelled by Chem 3D, versus a 2.9 Å interatomic distance for protons H-7' and H-8 in the case of **3.63b**. Furthermore, no NOE correlations were observed between the phenyl and pyrone groups, suggestive of the **3.63a** relative stereochemistry. Thus it was concluded that NOE data is not sufficiently conclusive to assign the relative geometry of substituted cyclobutanes such as **3.63**. The relative stereochemistry of dimer **3.63** has been previously reported in the literature as **3.63a** (Figure 3.18) being established by chemical oxidative conversion to the well described natural product *β*-truxinic acid (**3.65**).[115]



3.65

Cycloadduct **3.64** exhibited ^{1}H and ^{13}C NMR spectroscopic data that agreed with those as reported in the literature.[116,119] The observed ESI mass spectrum (*m/z* 479 $[M+Na]^{+}$) and subsequent high resolution data (*m/z* 479.1453, calcd for $C_{28}H_{24}O_6Na$ 479.1465) were in full agreement with the proposed structure. NOESY NMR correlations were observed between protons H-8, H-5' and H-7' indicating that these protons sit on the same face of the molecule. No NOESY correlation was observed between H-7 and any of these protons indicating a *trans* relationship between H-7 and H-8 as shown. The relative stereochemistry of **3.64** has been reported, determined by X-ray analysis, and is the same as shown in Figure 3.19.[114,123,124]

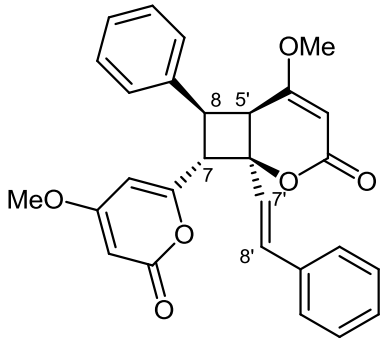
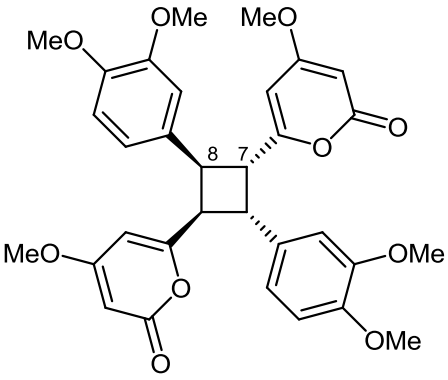
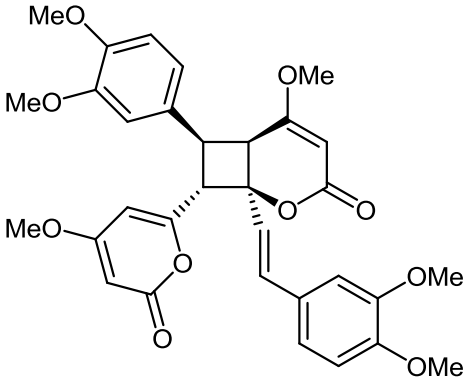


Figure 3.19 Relative configuration for cycloadduct **3.64**.

Photolysis of dimethoxyphenyl pyrone **3.47** gave two products **3.66** and **3.67** in yields of 14% and 35% respectively. Dimers **3.66** and **3.67** have been reported as natural products[118] along with the likely parent compound **3.47** which the authors speculated could have given rise to **3.66** and **3.67** as photo-artefacts during isolation. Previous studies of the photolysis of **3.47** have led to the characterisation of the dimer **3.67**.[130]



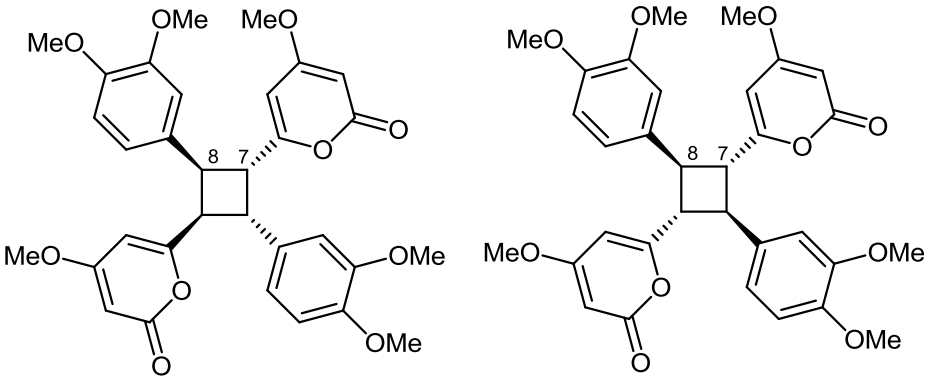
3.66



3.67

The dimer **3.66** exhibited ^{1}H and ^{13}C NMR spectral data in close agreement with those reported in the literature.[118] The ESI mass spectrum for **3.66** showed the expected molecular ion *m/z* 577. Fragmentation studies of **3.66**, using ESI MS/MS, gave only fragmentation of type F (Figure 3.17) which, while not being conclusive, suggested the head-to-tail structure of **3.66**. More conclusive was the analysis of the lineshape of the ^{1}H NMR signals for the cyclobutane protons where the triplet-type shape observed indicated that 3.66 was a type B dimer, as describe on pages 80-83. As with **3.63**, both *syn* (**3.66a**) and *anti* (**3.66b**) products could be expected, shown in Figure 3.20. The NOESY spectrum obtained for **3.66** exhibited correlations between H-7,7' and H-8,8'

which supports the *syn* configuration assigned (3.66a), however the spectrum did not show the expected correlations between the phenyl and pyrone moieties.



3.66a **3.66b**

Figure 3.20 Possible configurations for 3.66.

As noted in the case of 3.63, NOE correlations, or lack thereof, are unreliable in assigning the relative configuration around a cyclobutane ring. Closer inspection of the two potential relative stereochemistries shows that 3.66b is highly symmetric, H_A has the same angle and distance to each group in the molecule as $H_{A'}$ and equivalently H_B has the same angle and distance to each group in the molecule as $H_{B'}$. Consequently H_A and $H_{A'}$ are magnetically equivalent as are H_B and $H_{B'}$ and they therefore form an A_2B_2 spin system with $J_{AA} = J_{BB} = 0$ and $J_{AB} = J_{AB'}$. Using the chemical shift (δ_H 4.19 and 3.19 ppm) and frequency separation (ν_{AB} = 62 Hz), taken for the observed spectrum (Figure 3.21(a)), with a selection of potential J_{AB}'s (and equally $J_{AB'}$'s) NMR simulation software predicts lineshapes that do not match the observed lineshape. In particular the relative height of the lines making up each multiplet do not agree. A representative A_2B_2 simulation is given in Figure 3.21(b). However, if the spin-system is simulated as an AA'BB' system, with small $J_{AA'}$ and $J_{BB'}$ (0.2 and 0.3 Hz respectively) and mid-sized J_{AB} and $J_{AB'}$ (7.6 and 10.0 Hz respectively), a spectrum is predicted (Figure 3.21(c)) that closely matches the experimentally observed lineshape of **3.66**, and this inturn allows the unambiguous assignment of the relative stereochemistry for **3.66** as *syn* as shown.

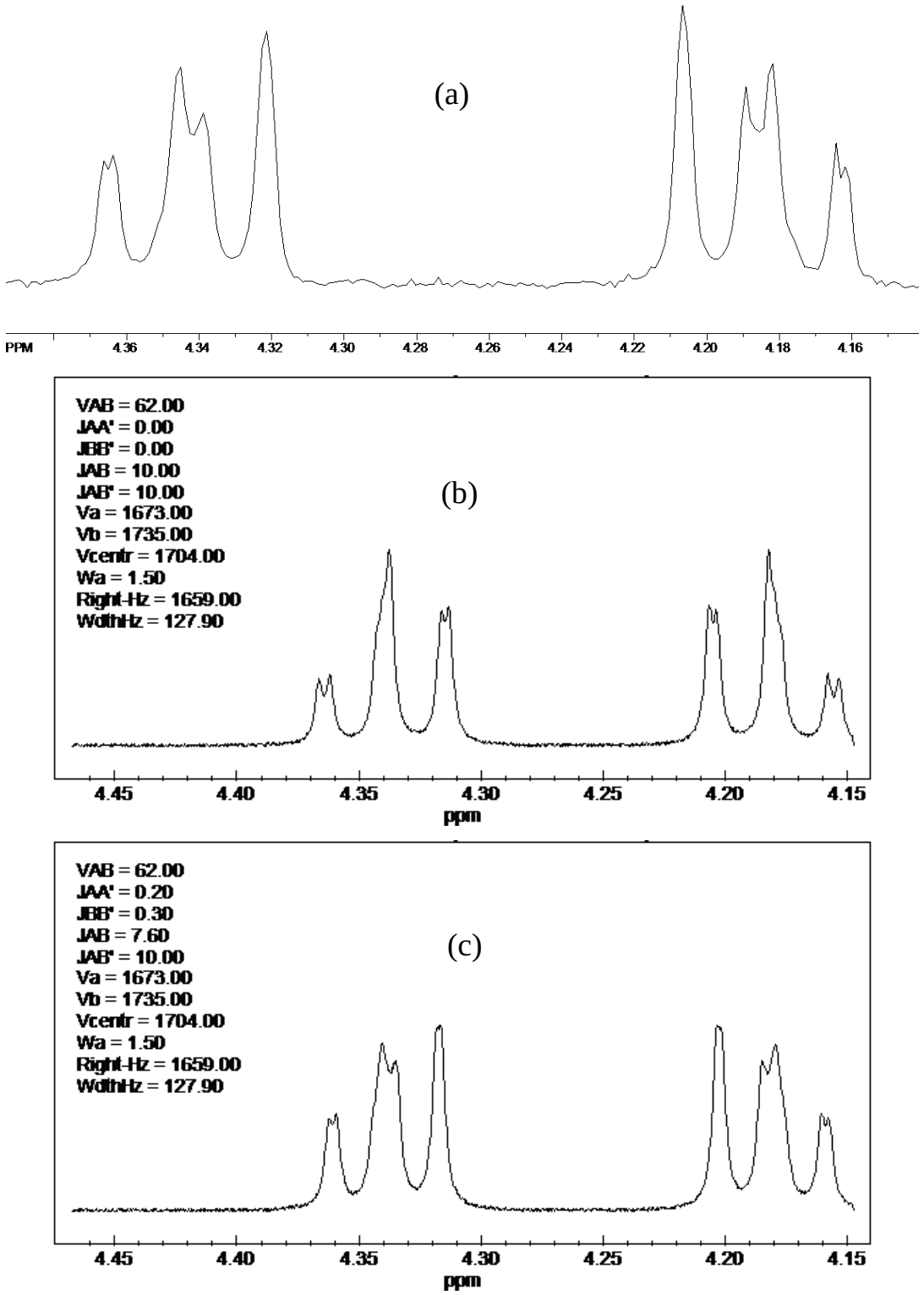


Figure 3.21 ^{1}H NMR spectra for **3.66** (a) observed spectrum (b) A_2B_2 spin simulation that most closely matches observed spectrum (c) An AA'BB' spin simulation that closely matches the observed spectrum.

Dimer **3.67** was similarly found to have ^{1}H and ^{13}C NMR spectral data that were in agreement with the reported literature.[118] The ESI mass spectrum of **3.67** was as

expected with the pseudo-molecular ion, $[M+H]^+$, of *m/z* 577 observed. The observed NOE correlations of **3.67** matched the equivalent correlations observed for the analogous dimer **3.64** and so the relative stereochemistry around the cyclobutane ring of **3.67** could be inferred to be the same as that reported in literature.[118]

Inspection of the X-ray crystal structure of the styryl pyrone **3.47** identified two orientations of adjacent layers in the crystal lattice (Figure 3.22). More detailed analysis of the inter-layer distances determined that one of the orientations is positioned well for [2 + 2] cycloaddition where Δ^5 of one molecule is aligned with Δ^7 of a second molecule with suitable inter-atomic distances of 3.6-3.7 Å. Previous studies have concluded that 3.6-4.1 Å is a typical intermolecular distance for cinnamic acid analogues to achieve [2 + 2] cycloaddition.[131] The second set of inter-atomic distances (5.8 Å) between Δ^7 of pyrone subunits are not suitable for [2 + 2] cycloaddition. This orientation indicates a preference for the formation of the type C dimer as observed. The isolation of type B dimer photoproduct is possibly due to the presence of an alternative crystal packing form in the amorphous solid used for the photoreaction.

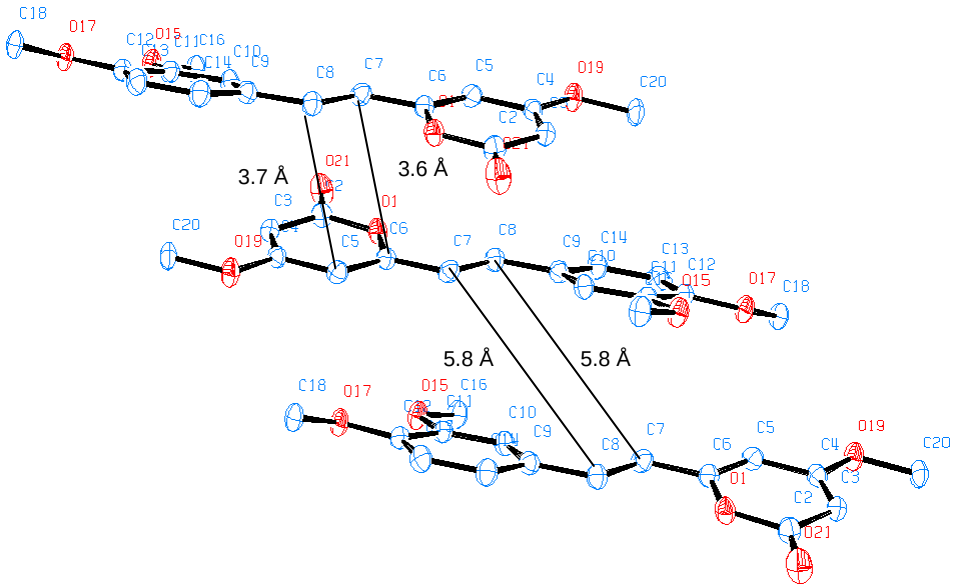
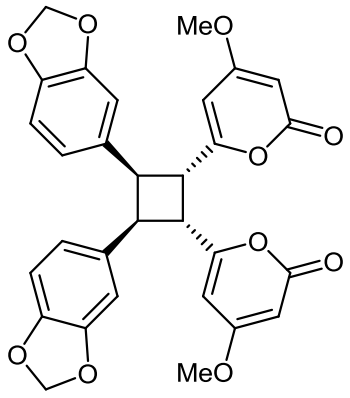


Figure 3.22 The X-ray crystal structure of **3.47**

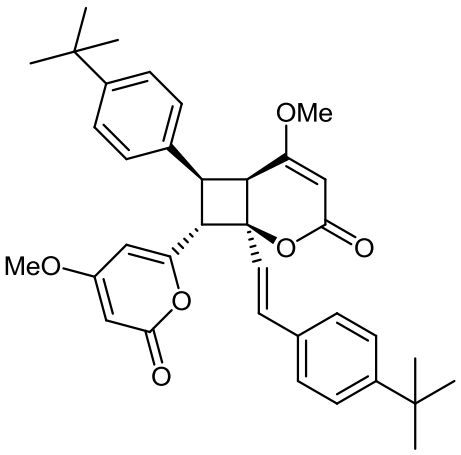
Photodimers of the natural product **3.36** have not previously been reported. The controlled photolysis of **3.36** gave low yields of dimer **3.68** with little indication in the ^{1}H NMR spectrum of any other photolysis product.



3.68

Electrospray mass spectral analysis (*m/z* 545) as well as ^{1}H and ^{13}C NMR analysis were consistent with the structure of **3.68**, with NMR spectral assignments being made with the aid of 2D NMR experiments. Two methods of analysis established the head-to-head nature of dimer **3.68**. First, the ^{1}H NMR line shape was in keeping with the established general line shape for head-to-head dimers as explained earlier (page 80-83). Secondly, the ESI-MS/MS spectrum identified a fragment of *m/z* 277.0690 (calcd for $C_{14}H_{13}O_6$ 277.0707) consistent with D type (Figure 3.17) fragmentation. The *syn* relative stereochemistry of **3.68** is assigned based on close simularities between the observed NMR data of **3.68** and **3.63**, including NOE correlations. This assignment however is not conclusive but is considered likely.

The photoreaction of a solid sample of synthetic styryl pyrone **3.46** proceeded with excellent specificity with only one significant product present, as indicated by the ^{1}H NMR spectrum. The specificity and reactivity observed mirrored the propensity of the stored material to dimerise. The ^{1}H and ^{13}C NMR spectroscopic data contained resonances that placed the product as a type C dimer, and this was fully assigned, with the aid of 2D NMR experiments, to structure **3.69**. The expected molecular ion was observed by HRESIMS (*m/z* 569) and NOESY NMR data for the cyclobutane ring and surrounding protons matched those observed for dimers **3.64** and **3.67** equivalently indicating the assigned relative stereochemistry.



3.69

Figure 3.23 shows the X-ray structure and packing of two adjacent molecules of styryl pyrone starting material **3.46** along with inter-atomic distances for carbons 5 and 6 on one molecule to carbons C-7 and C-8 on the other. The packing of the crystal shown indicates a clear alignment of Δ^5 of one molecule with the Δ^7 of a second molecule and the distances between these carbons, 3.5-3.9 Å, are appropriate for [2 + 2] cycloaddition.[131]

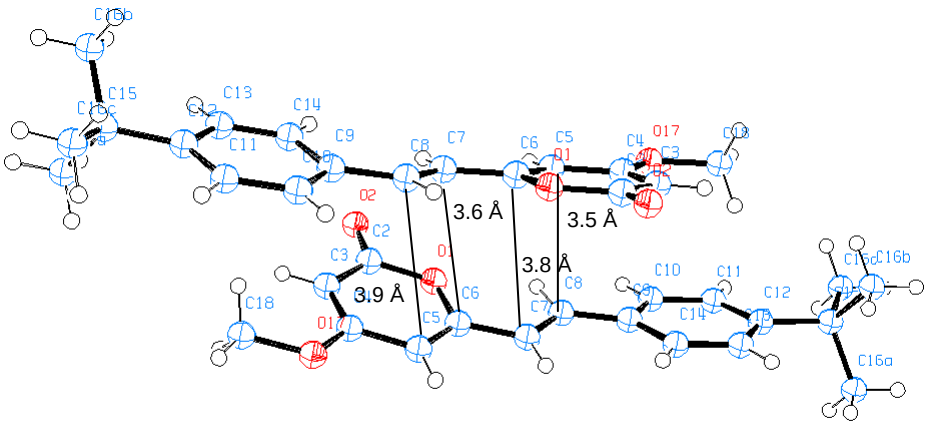
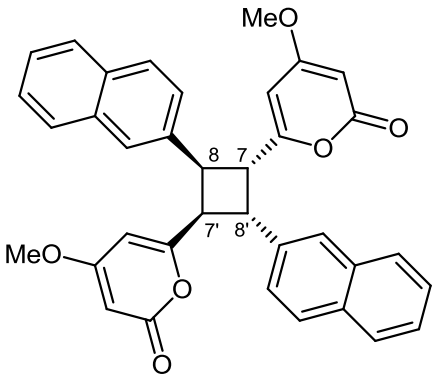


Figure 3.23 X-ray crystal structure of **3.46** showing the Δ^5 and Δ^7 intermolecular distances.

Photoreaction of 2-naphthyl pyrone **3.55** also proceeded in high yield to a single product. Analysis of the isolated photo-product by ^{1}H and ^{13}C NMR spectroscopy accompanied by 2D NMR studies concluded the structure to be dimer **3.70**.

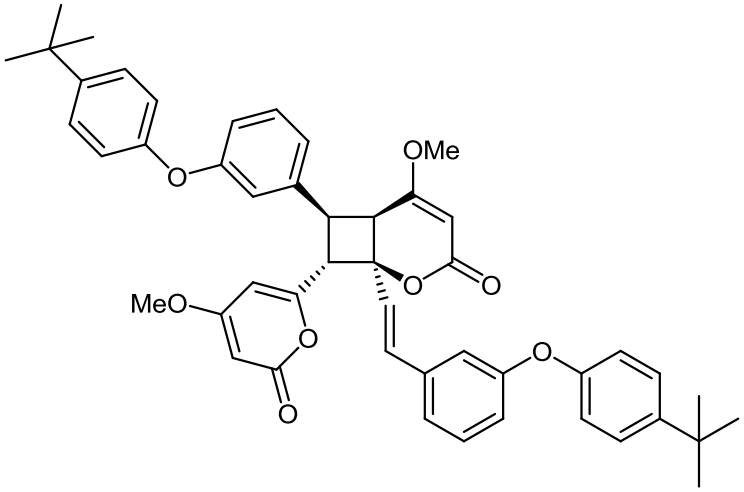


3.70

Mass spectrometric studies confirmed the molecule formula ((+)-HRESIMS *m/z* 557.1963 (calcd for $C_{36}H_{29}O_6$ 557.1959)). The head-to-tail of **3.70** orientation was determined by analysis of the ^{1}H NMR signal line shape, as explained earlier (page 80-83), and was further indicated by ESI-MS/MS fragmentation analysis which only elucidated fragmentation of F-type (Figure 3.17). The relative *syn* stereochemistry shown was based upon closer analysis of the observed ^{1}H NMR lineshape for the cyclobutane protons. As with dimer **3.66** the *anti* relative stereochemistry would form an A_2B_2 spin system for the cyclobutane protons and simulation shows that this cannot produce the lineshape observed for these protons. However simulation of an AA'BB' spin system (present in the *syn* relative stereochemistry) can and therefore **3.67** is assigned as having *syn* relative stereochemistry as shown.

It is interesting to note that the photoreaction of the isomeric 1-naphthyl pyrone **3.53** proceeded less readily and formed numerous unidentifiable side-products.

Finally, the photoreaction of styryl pyrone **3.44** gave one identifiable product, as determined by ^{1}H NMR spectroscopy. This type C dimer was analysed by ^{1}H, ^{13}C and 2D NMR studies and the structure assigned as **3.71**. High resolution mass spectrometric studies confirmed the molecular formula ((+)-HRESIMS *m/z* 753.3402 (calcd for $C_{48}H_{49}O_8$ 753.3422)) and NOE correlations were consistent with the relative stereochemistry shown and matched the equivalent correlations observed for **3.64**.

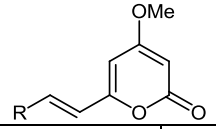


3.71

3.2.2 Biology

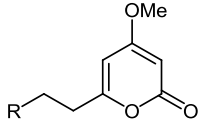
Presented in this section is data acquired by collaborators through the biological evaluation of compounds supplied by the author of the thesis. The evaluation of compounds against *M. tuberculosis* H_{37}Rv was performed by Drs Helena Boshoff and Clif Barry at the National Institute of Allergy and Infectious Diseases, NIH, USA. The evaluation of compounds against *Trypanosoma brucei rhodesiense, Leishmania donovani, Plasmodium falciparum* and an L6 rat skeletal myoblast cell line was performed by Drs Marcel Kaiser and Reto Brun, Department of Parasite Chemotherapy of the Swiss Tropical and Public Health Institute, Basel, Switzerland.

Table 3.8 Biological activities for Styryl-pyrones

entry	compound	R	M. tb.[a]	T.b.rhod.[b]	L.don.[c]	P. falc.[d]	L6[e]	SI rhod.[f]	SI don.[g]	SI falc.[h]
										
1	**3.39**	phenyl	>438	130.9	186.0	13.4	159.0	1.2	0.9	11.9
2	**3.42**	3-nitrophenyl	>366	>329	265.3	12.2	180.4	<0.548	0.7	14.8
3	**3.43**	3-phenoxy-phenyl	>312	6.8	28.3	4.9	13.1	1.9	0.5	2.7
4	**3.44**	3-(4-*tert*-butyl-phenoxy)-phenyl	>266	36.8	17.4	6.2	63.6	1.7	3.7	10.2
5	**3.45**	4-nitrophenyl	>366	38.8	21.8	6.0	191.6	4.9	8.8	31.7
6	**3.46**	4-*tert*-butyl-phenyl	>352	43.7	18.2	5.3	23.5	0.5	1.3	4.5
7	**3.36**	benzo[*d*][1,3]dioxolyl	>367	39.30	228.2	5.6	15.8	0.4	0.07	2.8
8	**3.47**	3,4-dimethoxyphenyl	>347	28.7	269.2	3.0	163.2	5.7	0.6	54.5
9	**3.48**	styryl	>393	147.2	221.7	6.7	192.5	1.3	0.9	28.6
10	**3.49**	4-dimethylaminostyryl	168.2	293.6	>303	7.2	25.4	0.09	<0.09	3.5
11	**3.50**	furanyl	>458	77.9	238.0	17.1	139.7	1.9	0.6	8.1
12	**3.53**	1-naphthyl	>359	16.5	30.7	4.6	111.7	6.8	3.6	24.1
13	**3.54**	dipyrone	>264	85.6	>238	8.6	>238	>2.8	nc[i]	>27.6
14	**3.55**	2-naphthyl	>359	2.4	>323	1.3	27.6	11.6	<0.09	21.6

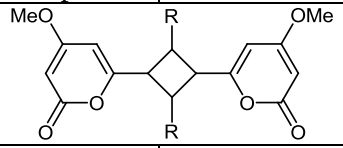
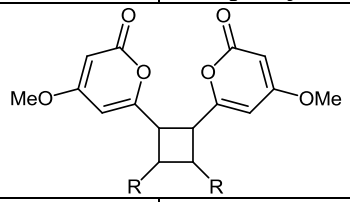
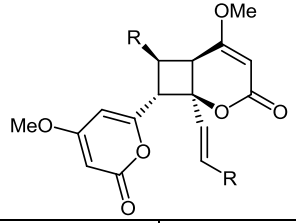
[a] *Mycobacterium tuberculosis* $H_{37}Rv$ grown in 7H9 medium, MIC (μM); [b] *Trypanosoma brucei rhodesiense* (strain STIB 900), trypomastigote stage. IC_{50} (μM); [c] *Leishmania donovani* (strain MHOM-ET-67/L82), amastigote/axenic stage. IC_{50} (μM); [d] *Plasmodium falciparum* (strain K1), IEF stage. IC_{50} (μM); [e] L6 rat skeletal myoblast cell line. IC_{50} (μM); [f] selectivity index for *Trypanosoma brucei rhodesiense* against L6; [g] selectivity index for *Leishmania donovani* against L6; [h] selectivity index for *Plasmodium falciparum* against L6, [i] nc, not calculable.

Table 3.9 Biological activities for alkyl-pyrones

entry	compound	R	M. tb.[a]	T.b.rhod.[b]	L.don.[c]	P. falc.[d]	L6[e]	SI rhod.[f]	SI don.[g]	SI falc.[h]
										
1	**3.33**	benzo[*d*][1,3]dioxolyl	>365	136.5	205.5	6.2	178.5	1.3	0.9	28.6
2	**3.56**	phenyl	>434	281.2	209.4	>21.7	290.8	1.0	1.4	<13.4
3	**3.57**	3-(4-*tert*-butyl-phenoxy)-phenyl	132.1	43.0	12.9	7.2	49.9	1.2	3.9	6.9
4	**3.58**	4-*tert*-butyl-phenyl	174.6	54.1	15.2	11.1	95.2	1.8	6.3	8.6
5	**3.59**	3,4-dimethoxyphenyl	>344	186.8	224.5	>17.2	>310	>1.7	>1.4	nc[i]
6	**3.60**	1-naphthyl	267.6	77.8	37.3	>17.8	109.8	1.4	2.9	<6.2
7	**3.61**	dipyrone	>262	>235	>235	3.6	49.5	<0.2	<0.2	13.7
8	**3.62**	2-naphthyl	178.4	50.5	35.2	15.5	90.5	1.8	2.6	5.8

[a] *Mycobacterium tuberculosis* H_{37}Rv grown in 7H9 medium, MIC (μM); [b] *Trypanosoma brucei rhodesiense* (strain STIB 900), trypomastigote stage. IC_{50} (μM); [c] *Leishmania donovani* (strain MHOM-ET-67/L82), amastigote/axenic stage. IC_{50} (μM); [d] *Plasmodium falciparum* (strain K1), IEF stage. IC_{50} (μM); [e] L6 rat skeletal myoblast cell line. IC_{50} (μM); [f] selectivity index for *Trypanosoma brucei rhodesiense* against L6; [g] selectivity index for *Leishmania donovani* against L6; [h] selectivity index for *Plasmodium falciparum* against L6, [i] nc, not calculable.

Table 3.10 Biological activities for pyrones photodimers

entry	compound	R	M. tb.[a]	T.b.rhod.[b]	L.don.[c]	P. falc.[d]	L6[e]	SI rhod.[f]	SI don.[g]	SI falc.[h]
										
1	**3.66**	3,4-dimethoxyphenyl	>173	6.7	65.8	3.1	21.6	3.2	0.3	7.0
2	**3.70**	2-naphthyl	>180	3.8	9.5	2.3	95.2	25.1	10.0	42.1
										
3	**3.63**	phenyl	>219	15.4	15.8	7.1	>197	>12.8	>12.5	>27.7
4	**3.68**	benzo[*d*][1,3]dioxolyl	>184	nd	nd	nd	nd	nd	nd	nd
										
5	**3.64**	phenyl	>219	16.6	13.8	3.8	51.3	3.1	3.7	13.4
6	**3.67**	3,4-dimethoxyphenyl	>173	62.4	79.1	1.5	115.6	1.9	1.5	74.9
7	**3.69**	4-*tert*-butyl-phenyl	>176	23.1	3.1	1.7	133.2	5.8	42.8	78.8
8	**3.71**	3-(4-*tert*-butyl-phenoxy)-phenyl	>133	>120	57.8	4.4	>120	nc[j]	>2.1	>27.2

[a] *Mycobacterium tuberculosis* H_{37}Rv grown in 7H9 medium, MIC (μM); [b] *Trypanosoma brucei rhodesiense* (strain STIB 900), trypomastigote stage. IC_{50} (μM); [c] *Leishmania donovani* (strain MHOM-ET-67/L82), amastigote/axenic stage. IC_{50} (μM); [d] *Plasmodium falciparum* (strain K1), IEF stage. IC_{50} (μM); [e] L6 rat skeletal myoblast cell line. IC_{50} (μM); [f] selectivity index for *Trypanosoma brucei rhodesiense* against L6; [g] selectivity index for *Leishmania donovani* against L6; [h] selectivity index for *Plasmodium falciparum* against L6, [i] nc, not calculable.

3.2.2.1 Antitubercular data

Evaluation of the compound library against *Mycobacterium tuberculosis* $H_{37}Rv$ identified only four active pyrones active, with weak high micromolar potency. Entry 10 (**3.49**, Table 3.8) was active with an MIC of 168.2 μM as well as entries 3 (**3.57**), 4 (**3.58**), 6 (**3.60**) and 8 (**3.62**) (Table 3.9) with MICs of 132.1, 174.6, 267.6 and 178.4 μM respectively. Of the four compounds, **3.49** was the only styryl pyrone, with **3.57**, **3.58**, **3.60** and **3.62** being alkyl pyrones. It can be noted that the analogous styryl counterparts to these alkyl compounds were not measurably active in this assay. This leads to the conclusion that the antitubercular activity of these compounds is greater for alkyl pyrones than the corresponding styryl compounds and further that the dimer pyrones were relatively less active. It can be noted that compounds **3.39** and **3.33** were reported with activity of 140 and 15 μM respectively against *M. tuberculosis* $H_{37}Rv$ strain,[34] however in this study these compounds were found to be inactive, with MIC > 440 μM and > 370 μM respectively. The primary difference in these assays is the growth medium used, with the current data being run in an assay format that is rich in bovine serum albumin (BSA). BSA is a non-specific binding protein that can sequester various compounds and thus limiting their bioavailability. This process is thought to be responsible for the relative drop in observed activity.

3.2.2.2 Antiparasitic screening

The pyrones were evaluated against three parasites: *Trypanosoma brucei rhodesiense*, *Leishmania donovani* and *Plasmodium falciparum*. The activity of the pyrones tested was strongest against *P. falciparum*, with the exception of alkyl pyrones **3.56**, **3.59** and **3.60**. These compounds had activity against *P. falciparum* below the detection limit for the assay, but potentially still higher than the activities measured against *T. b. rhodesiense* and *L. donovani*. This trend shows a general selectivity of this type of pyrone towards *P. falciparum* versus the other parasites.

The styryl-type pyrones (Table 3.8) possessed modest anti-parasitic activity towards *P. falciparum* with IC_{50} values ranging from 1.3 μM (**3.55**) through to 17.4 μM (**3.50**) and a medium of 6.0 μM around which most activities cluster. It can be noted that the

simplest/smallest members of the styryl library have the lowest molar activities with **3.39** and **3.50** (having a phenyl and a furanyl group respectively) which have activities of 13.4 and 17.4 μM, respectively. The alkyl type pyrones (Table 3.9) are less active against *P. falciparum* than their styryl counterparts (Table 3.8), most notably is the 12-fold difference in activity found for the 2-naphthyl compounds **3.55** and **3.62**. The exception to this trend is the dipyrone **3.61**, most likely due to the very much higher solubility of **3.61** compared to the styryl parent compound **3.54**. Interestingly, this trend in activity is in contrast to the observed trend for these compounds against *Mycobacterium tuberculosis*. When compared to their starting monomers, the two head-to-tail photodimers (Table 3.10) showed molar activity against *P. falciparum* that was identical for **3.66** and slightly reduced (IC_{50} 1.3 cf. 2.3 μM) for **3.70**. The head-to-head dimer **3.63**, however, showed an increase in activity when compared to its original monomer (IC_{50} 7.1 cf. 13.4 μM). Of greater interest are the type C dimers. These interesting structures all presented higher molar activity against *P. falciparum* when compared to their starting monomers. Furthermore, **3.64** and **3.67** were more active than their head-to-head/tail isomers (**3.63** and **3.66**, respectively).

The activity against *Leishmania donovani* observed for the pyrone library was relatively lower than and more variable than that observed against *Plasmodium falciparum*. For the styryl type pyrones the activity against *L. donovani* ranged from 17.4 μM (**3.44**) to >323 μM (**3.55**) with their being two groups with either IC_{50} ~15-30 μM activity (**3.43-3.46**, **3.53**) or IC_{50} >180 μM. Of interest are the isomeric pairs (1-naphthyl (**3.53**) and 2-naphthyl (**3.55**), and 3-nitrophenyl (**3.42**) and 4-nitrophenyl (**3.45**)) where one member in each pair is notably more active (*ie*. **3.53** and **3.45**). When considering the 6-alkyl-pyrones, the activities against *L. donovani* closely follow those found for their styryl counterparts with the exception of 2-naphthyl alkyl pyrone **3.62** which has an IC_{50} of 35.2 μM compared with the IC_{50} > 323 μM for **3.55**. This trend is in contrast to that observed for both *P. falciparum*, where activities were lower for alkyls, and *Mycobacterium tuberculosis*, where the activities were found to be greater for the alkyls. Six of the seven tested dimers showed > 3-fold increase in activity against *L. donovani* but the largest dimer (**3.71**) showed a ~3-fold decrease in activity. Of interest is the similarity in activity of isomeric dimers **3.66** and **3.67** along

with **3.63** and **3.64** which is distinct from the trend of enhanced activity for type C dimers against *P. falciparum*.

The IC_{50}s observed against *Trypanosoma brucei rhodesiense* were the most variable of the three parasites tested. Activities against *T. b. rhodesiense* for styryl pyrones ranged form 2.4 μM (**3.55**) to > 329 μM (**3.42**) and were spread fairly evenly over this range of activities. All alkyl pyrones tested were found to have less activity against *T. b. rhodesiense* than their styryl counterparts which was in keeping with the general trend observed for anti-*Plasmodium falciparum* activity. The activities against *T. b. rhodesiense* found for the photodimers tested were also quite variable ranging from 3.8 μM to >120 μM. Importantly the activity relative to the starting styryl pyrone varied significantly with three examples with 2-4-fold reduced activity (**3.70**, **3.67** and **3.71**) and four examples with 2-8-fold increased activity (**3.66**, **3.63**, **3.64** and **3.69**). The head-to-head/tail dimers were all more active than the type C dimers.

3.2.2.3 Selectivity and Cytotoxicity

Cytotoxicity was measured against an L6 rat skeletal myoblast cell line. The cytotoxicity of this class of compounds is too low to be of interest for anticancer development. Low cytotoxicity, however, is desirable when the compound also presents moderate to high activity against a pathogenic species. The selectivity index is the ratio of the cytotoxicity of the compound (IC_{50} against L6) to the activity of the target species (IC_{50} of each parasite).

While the activity of the series of compounds against *Mycobacterium tuberculosis* was too low to discuss selectivity, modest selectivity (>10) was found for examples against each of the parasites evaluated. For *Trypanosoma brucei rhodesiense*, selectivity was observed for the styryl type 2-nathphyl pyrone **3.55** (SI = 12), head-to-tail 2-naphthyl dimer **3.70** (SI = 25) and phenyl head-to-head dimer **3.63** (SI > 13). Additionally **3.54** and **3.59** had cytotoxicity levels that were below the detection limit and were therefore potentially highly selectivity.

Selectivity against *Leishmania donovani* was less common and of the styryl- and alkyl-type pyrones, only **3.59** had a reasonable selectivity with low cytotoxicity (IC_{50} > 310 μM). As with *Trypanosoma brucei rhodesiense*, phenyl head-to-head dimer **3.63** showed selectivity (SI >13) and the low cytotoxicity of **3.71** (IC_{50} > 50 μM) may indicate that **3.71** is also selective. The most selective compound quantified was type C dimer **3.69**, which had a selectivity index of 43.

The selectivity of these pyrones against *Plasmodium falciparum* is far more significant than for the other two parasites, with sixteen examples showing some measure of selectivity. Among the styryl pyrones, eight of the fourteen examples (**3.39**, **3.42**, **3.45**, **3.47**, **3.48**, **3.53**, **3.54**, and **3.55**) had significant selectivity with **3.47** having the highest (SI = 54). Also of note is the selectivity of dipyrone **3.54** (SI >28) due to low cytotoxicity (IC_{50} > 238 μM). In contrast, only two of the eight alkyl examples tested had significant selectivity, **3.33** (SI = 29) and **3.61** (SI = 14). The dimers were the most selective of the classes tested with six of the seven dimers having significant selectivity against *P. falciparum* and the less selective example (**3.66**) still having a SI of 7. With the exception of **3.66**, the dimers were all more selective than their precursor monomers. The largest selectivities measured of any of the pyrones tested was of type C dimers **3.67** and **3.69** with selectivities of 75 and 79, respectively. Also of note are dimers **3.63** and **3.71**, SI >28 and SI >27 respectively, which had low cytotoxicity (IC_{50} > 197 μM and > 120 μM, respectively).

3.3 Conclusions

Libraries of 6-styryl-4-methoxy-2-pyranones and 6-alkyl-4-methoxy-2-pyranones were prepared and characterised. Photochemistry of several examples of 6-styryl-4-methoxy-2-pyranones was explored and eight photodimers, including four novel dimers, were purified and characterised. Biological evaluation of these pyrones by collaborators showed little indication of anti-*Mycobacterium tuberculosis* activity, in contrast to reported examples, but did indicate selectivity of these compounds against several parasites. A few examples were found to be significantly selective against *Trypanosoma brucei rhodesiense* and *Leishmania donovani* but far more pyrones

were found to be selective against *Plasmodium falciparum*. Most notably, pyrone dimers were found to have good to excellent selectivity against *P. falciparum*.

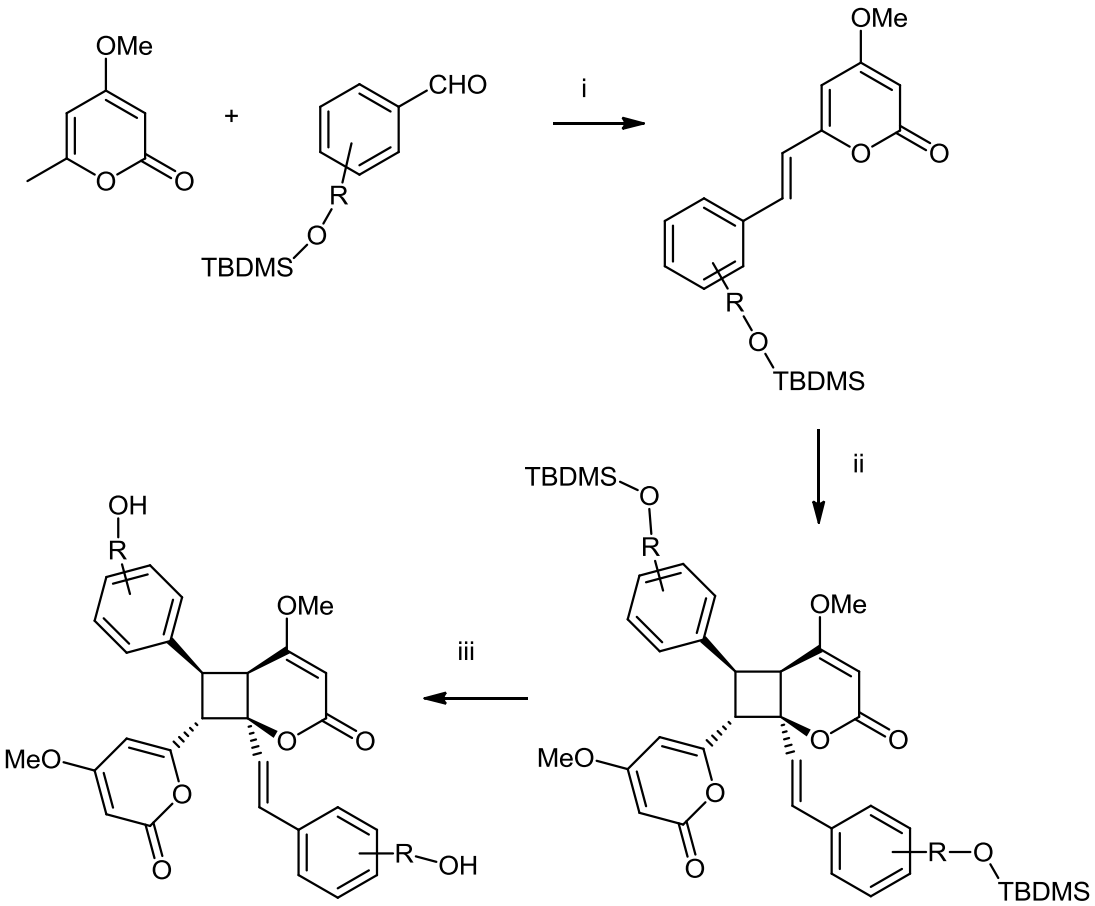
3.4 Future work

Investigations of this class of compounds could be further expanded by the demethylation of the pyrone ring to target two additional sets of compounds (Figure 3.24). These examples will more closely mimic the pseudopyronines and enable further exploration the biological activity of this compound class.

Figure 3.24 Demethylated targets for future studies.

Further investigation of photodimerisation could be extended to include all examples of potential monomers made as well as optimisation of the conditions used to reduce decomposition products and increase product yield. This is most likely achieved by using a lower intensity light source (either by greater distance from the light source or a less intense light source) over a longer period of time. In addition, significant yield increases could be achieved by further developing chromatographic methodology possibly by moving to different solvent systems, where appropriate, or even utilising a silica-bonded solid phase.

Further to this, investigation into the formation of readily modifiable dimers could be undertaken. As demonstrated earlier, the presence of a *tert*-butyl group directs the crystalline packing of styryl pyrones so that exposure to light yields only type C dimers. The incorporation of a base stable readily cleavable *tert*-butyl containing group, such as TBDMS, would allow the preparation of this basic scaffold. This scaffold in turn could be modified to produce numerous analogues (Scheme 3.4).



Scheme 3.4 Proposed general synthesis of type C dimers.
Reagents and conditions: i) $Mg(MeO)_2$, MeOH; ii) hv; iii) HCl.

Chapter 4 Experimental

4.1 General laboratory procedures

Low resolution mass spectra were recorded on either a VG-7070 mass spectrometer operating at a nominal accelerating voltage of 70 eV or a Bruker micrOTOF-Q II mass spectrometer. High resolution mass spectra were recorded at a nominal resolution of 5000 or 10000 as appropriate. All spectra were obtained using EI, FAB, CI, or ESI ionisation techniques using perfluorokerosene, 3-nitrobenzyl alcohol or polyethylene glycol as the internal standards. Infrared spectra were recorded as dry films on sodium chloride or ATR crystal and recorded with a Perkin Elmer Spectrum One Fourier Transform infrared spectrometer with Universal ATR Sampling Accessory. ν_{max} are expressed cm^{-1}. Melting points were recorded on a Electrothermal melting point apparatus and are uncorrected. All NMR spectra were recorded either using a Bruker Avance 300 MHz spectrometer operating at 300.13 MHz for ^{1}H nuclei and 75.47 MHz for ^{13}C nuclei or a Bruker DRX 400 MHz spectrometer operating at 400.13 MHz for ^{1}H nuclei and 100.62 MHz for ^{13}C nuclei or a Bruker Avance 600 MHz spectrometer operating at 600.17 MHz for ^{1}H nuclei and 150.93 MHz for ^{13}C nuclei. Chemical shifts are expressed in parts per million (ppm) relative to the residual non-deuterated solvent in ^{1}H NMR and to deuterated solvent in ^{13}C NMR ($CHCl_3$: ^{1}H 7.25, ^{13}C 77.0 ppm; DMSO-d_6: ^{1}H 2.50, ^{13}C 39.4 ppm; MeOH-d_4: ^{1}H 3.30, ^{13}C 49.0 ppm, D_2O referenced to an internal standard as stated in each case). For ^{1}H NMR, the data are quoted as position (δ), relative integral, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, sep = septet, m = multiplet, b = broad, obsc = obscured), coupling constant (*J*, Hz), and assignment to the atom. The ^{13}C NMR data are quoted as position (δ) and assignment to the atom. Assignments are based on 1- and 2-dimensional experiments and analogue comparisons. Standard Bruker pulse sequences were utilised. Pressurised (flash) column chromatography was performed on either Kieselgel 60 0.063 - 0.200 mesh (Merck) silica gel or on LiChroprep® RP-18 (40-63 μm) bonded phase silica gel. LH20 colomn chromotography was performed on Sephadex® LH-20. Analytical thin layer chromatography (TLC) was carried out on 0.2 mm thick plates of Kieselgel F_{254} (Merck).

Reactions were heated by immersion in oil or by use of DrySyn^{tm} MULTI reaction block kit while the temperature was taken from a thermometer touching the bottom of the pyrex bath.

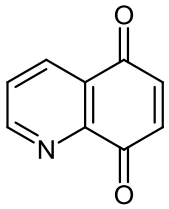
All solvents used were analytical grade or better and/or purified according to standard procedures. Chemical reagents used were either purchased from standard chemical suppliers and used as purchased or prepared according to literature procedures and purified to match the reported physical and spectral data.

Microanalyses were carried out by the Campbell Laboratory, University of Otago, Dunedin, New Zealand.

Compound purity was determined by reverse-phase HPLC (Waters 600 HPLC photodiode array system, Alltech Econosphere C8, 3 μm, 7 x 33 mm, H_2O (0.05% TFA) to MeCN over 8 min at 2.0 mLmin^{-1} and monitoring at 254 nm or at the wavelength stated.

4.2 Experimental details for Chapter 2

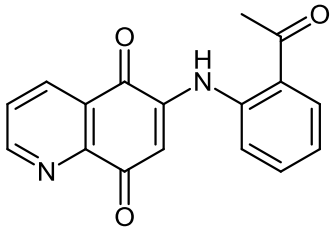
Quinoline-5,8-dione (**2.23**):



Quinoline-5,8-dione **2.23** was prepared following the procedures outlined by Pratt and Drake.[132] 5-Amino-8-hydroxyquinoline was made in this manner from crude 5-nitroso-8-hydroxyquinoline previously made within the group. This in turn was oxidised to quinoline-5,8-dione in 10 g portions as required, with the dione being used in further reactions immediately after being made and without purification.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.02 (1H, dd, *J* = 4.6, 1.7 Hz), 8.39 (1H, dd, *J* = 7.9, 1.7 Hz), 7.12 (1H, d, *J* = 10.4 Hz), 7.03 (1H, d, *J* = 10.4 Hz).

6-[(2-Acetylphenyl)amino]-5,8-quinolinedione (**2.24**):

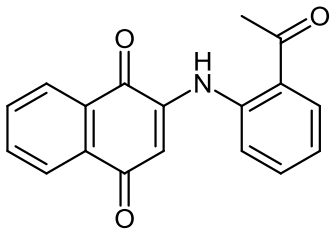


This procedure was completed following literature examples.[83,133] To a solution of quinoline-5,8-dione (1.53 g, 9.7 mmol) and cerium trichloride heptahydrate (1.79 g, 4.81 mmol) in ethanol (35 mL) was added 2'-amino acetophenone (1.2 mL, 9.9 mmol) and stirred for 18 hr. The reaction mixture was filtered and the solid washed with ice cold ethanol. This afforded red crystalline material **2.24** (1.89 g, 67%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 11.31 (1H, br, NH), 9.00 (1H, br), 8.46 (1H, d, *J* = 7.6 Hz), 7.96 (1H, d, *J* = 7.8 Hz), 7.64-7.55 (3H, m), 7.19 (1H, t, *J* = 7.3 Hz), 6.90 (1H, s), 2.68 (3H, s, Me); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 201.5, 182.7, 181.5, 155.1, 148.5,

143.6, 139.8, 134.7, 134.2, 132.4, 126.7, 125.7, 123.4, 120.7, 107.1, 28.4 one carbon unobserved; NMR data consistent with that previously reported in the Copp group.[87] EIMS *m/z* 292 $[M]^+$; HREIMS *m/z* 292.08443 (calcd for $C_{17}H_{12}N_2O_3$ 292.08479).

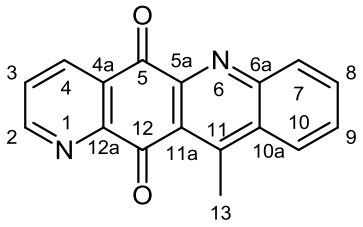
2-[(2-Acetylphenyl)amino]-1,4-naphthalenedione (**2.27**):



2-[(2-Acetylphenyl)amino]-1,4-naphthalenedione **2.27** was prepared following a literature procedure.[55] To a solution of 1,4-napthoquinone (2.62 g, 16.6 mmol) and cerium trichloride heptahydrate (3.136 g, 8.42 mmol) in ethanol (340 mL) was added 2'-amino acetophenone (2.0 mL, 16.5 mmol) and stirred for 48 hr. The reaction mixture was filtered and the solid washed with ice cold ethanol. The combined mother liquor and washings were reduced in volume in vacuo and the reaction mixture stirred for a further 48 hrs. The collection process was repeated a further 3 times to afford red crystalline material **2.27** (2.92 g, 61%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 11.26 (1H, br, NH), 8.15 (1H, dd, *J* = 7.6, 1.3 Hz), 8.09 (1H, dd, *J* = 7.8, 1.0 Hz), 7.94 (1H, dd, *J* = 8.0, 1.2 Hz), 7.73 (1 H, td, *J* = 7.5, 1.4 Hz), 7.70-7.54 (3H, m), 7.16 (1H, td, *J* = 7.5, 1.1 Hz), 6.72 (1H, s), 2.67 (3H, s, Me); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 201.2, 184.5, 181.7, 144.0, 140.3, 134.6, 134.1, 132.9, 132.6, 132.3, 130.7, 126.8, 126.0, 125.7, 122.8, 120.6, 106.5, 28.4; NMR data consistent with that previously determined in the Copp group.[87] (It should be noted that the literature has a discrepancy whereby one doublet reported at 7.06 ppm should be actually be reported at 7.60).[55,134] EIMS *m/z* 291 $[M]^+$; HREIMS *m/z* 291.08943 (calcd for $C_{18}H_{13}NO_3$ 291.08954).

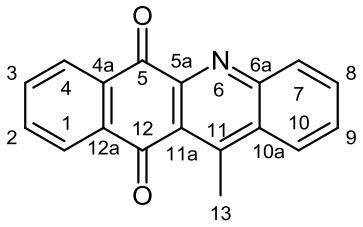
11-Methylpyrido[2,3-b]acridine-5,12-dione (**2.9**):



11-Methylpyrido[2,3-b]acridine-5,12-dione **2.9** was prepared following Bracher's methodology.[83] To a suspension of adduct **2.24** (1.85 g, 6.3 mmol) in glacial acetic acid (100 mL) was added conc sulphuric acid (10 mL) at 0ºC. The mixture was refluxed for 20 min and cooled to room temperature. The mixture was poured onto ice water (800 mL) and neutralised with conc ammonia. The mixture was extracted with dichloromethane (5 x 100 mL) and the combined organic layers were washed with water (1 x 200 mL) and dried with magnesium sulphate. The organic solution was then dried in vacuo. This afforded tan solid **2.9** (1.30 g, 75%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.15 (1H, dd, *J* = 4.6, 1.7 Hz), 8.72 (1H, dd, *J* = 7.9, 1.7 Hz), 8.45 (1H, d, *J* = 8.1 Hz), 8.39 (1H, d, *J* = 8.5 Hz), 7.93 (1 H, ddd, *J* = 6.9, 6.9, 1.1 Hz), 7.79 (1H, m), 7.75 (1H, m), 3.33 (3H, s, H-13); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 183.4, 181.9, 155.7, 152.8, 150.3, 148.7, 147.6, 135.8, 132.8, 132.4, 130.1, 129.9, 127.9, 125.6, 16.7, 2 carbons obscured at 129.9 and 125.6 ppm; NMR data consistent with previously published.[83,88] EIMS *m/z* 274 $[M]^+$; HREIMS *m/z* 274.07404 (calcd for $C_{17}H_{10}N_2O_2$ 274.07423).

11-Methylbenz[b]acridine-5,12-dione (**2.10**):



11-Methylbenz[b]acridine-5,12-dione **2.10** was prepared following a literature procedure.[55] To a suspension of adduct **2.27** (1.98 g, 6.8 mmol) in glacial acetic acid (65 mL) was added conc sulphuric acid (6.5 mL) at 0ºC. The mixture was refluxed for 40 min and cooled to room temperature. The mixture was poured onto ice water (600

mL) and neutralised with conc ammonia. The mixture was extracted with dichloromethane (5 x 100 mL) and the combined organic layers were washed with water (1 x 200 mL) and dried with magnesium sulphate. The organic solution was then dried in vacuo. This afforded tan solid **2.10** (1.00 g, 54%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.39 (1H, dd, *J* = 8.4, 0.7 Hz, H-7), 8.35 (1H, m, H-4), 8.30 (1H, dd, *J* = 8.7, 0.9 Hz, H-10), 8.26 (1H, m, H-1), 7.85 (1 H, ddd, *J* = 6.9, 6.8, 1.1 Hz, H-8), 7.80 (1H, m, H-2), 7.77 (1H, m, H-3), 7.70 (1H, ddd, *J* = 6.9, 6.9, 1.3 Hz, H-9), 3.24 (3H, s, H-13); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 185.0 (C-12), 182.1 (C-5), 151.8 (C-11), 148.5 (C-6a), 148.3 (C-5a), 135.3(C-12a), 134.6 (C-3), 133.9 (C-2), 133.3 (C-4a), 132.3 (C-7, C-8), 129.7 (C-10a), 129.4 (C-9), 127.4 (C-1, C-4), 125.3 (C-11a, C-10), 16.5 (C-13); NMR data consistent with previously published;[55] EIMS *m/z* 273 $[M]^+$; HREIMS *m/z* 273.07880 (calcd for $C_{18}H_{11}NO_2$ 278.07898).

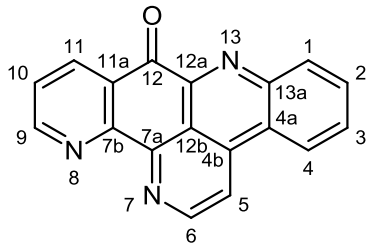
General method for preparation of compounds **2.5**, **2.11**-**14**, **2.25**, **2.28**-**2.32**, **2.37**, **2.43**-**45**:

A mixture of quinone tetracyle (**2.9** or **2.10**) (0.4 mmol), ammonium chloride (12.0 mmol) and the appropriate aldehyde (2.0 mmol) in acetic acid (50 mL) was refluxed under nitrogen for 50 min. After cooling, the reaction mixture was poured onto water, made basic with conc ammonia, and extracted with dichloromethane. The combined organic layers were washed with water, dried with magnesium sulfate and concentrated in vacuo. Purification procedures for individual compounds follow.

Modified workup:

To limit the presence of ammonium ions, which proved to be difficult to remove from some compounds, the neutralisation of acetic acid was conducted with sodium bicarbonate with the rest of work up being maintained as above.

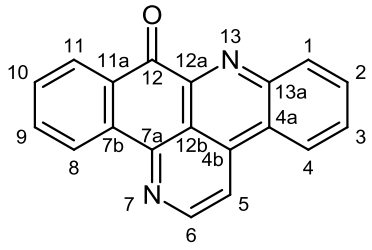
Ascididemin (**2.5**):



Prepared according to the general procedure using quinone **2.9** (109 mg, 0.40 mmol) and paraformaldehyde (61 mg, 2.0 mmol). Reaction workup (standard conditions), was followed by precipitation from dichloromethane with methanol and reduced pressure to afford **2.5** as a yellow solid (23 mg, 20%).

m.p. 303-305 ºC (lit.[32] > 300 ºC); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.30 (1H, d, *J* = 5.8 Hz), 9.19 (1H, dd, *J* = 4.7, 1.8 Hz), 8.81 (1H, dd, *J* = 7.9, 1.9 Hz), 8.72 (1H, dd, *J* = 8.1, 1.2 Hz), 8.66 (1H, dd, *J* = 8.2, 1.2 Hz), 8.57 (1H, d, *J* = 5.7 Hz), 8.04 (1H, ddd, *J* = 7.3, 7.1, 1.5 Hz), 7.97 (1H, ddd, *J* = 7.3, 6.9, 1.4 Hz), 7.68 (1H, dd, *J* = 8.0, 4.7 Hz); EIMS *m/z* 283 $[M]^+$; HREIMS *m/z* 283.07410 (calcd for $C_{18}H_9N_3O$ 283.07456). Data consistent with previously collected within the group and previously published.[87]

8-Deaza-ascididemin (**2.11**):

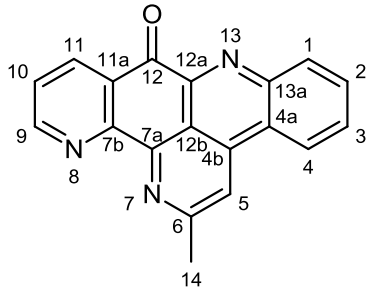


Prepared according to the general procedure using quinone **2.10** (109 mg, 0.40 mmol) and paraformaldehyde (61 mg, 2.0 mmol).ref. Reaction workup (standard conditions) was followed by flash column chromatography, (silica gel, dichloromethane), this afforded **2.11** as a yellow solid (50 mg, 44%).

m.p. 269-271 ºC (lit.[55] 260-262 ºC); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.96 (1H, d, *J* = 5.7 Hz, H-6), 8.78 (1H, d, *J* = 8.0 Hz, H-8), 8.53 (2H, d, *J* = 8.3 Hz, H-1, H-4), 8.43 (1H, d, *J* = 7.8 Hz, H-11), 8.29 (1H, d, *J* = 5.7 Hz, H-5), 7.92 (1H, td, *J* = 7.6, 1.2 Hz,

H-2), 7.82 (1H, td, *J* = 7.6, 1.0 Hz, H-3), 7.78 (1H, td, *J* = 7.6, 1.0 Hz, H-9), 7.64 (1H, td, *J* = 7.5, 1.0 Hz, H-10); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 182.0 (C-12), 150.4 (C-7a), 148.8 (C-6), 146.7 (C-12a), 145.7 (C-13a), 137.7 (C-4b), 136.0 (C-7b), 134.8 (C-9), 132.9 (C-1), 132.4 (C-11a), 131.5 (C-2), 131.1 (C-10), 130.2 (C-3), 128.6 (C-11), 125.6 (C-8), 123.3 (C-4a), 122.7 (C-4), 116.9 (C-12b), 115.3 (C-5); NMR data consistent with previously published;[55] EIMS *m/z* 282 $[M]^+$; HREIMS *m/z* 282.07914 (calcd for $C_{19}H_{10}N_2O$ 282.07931).

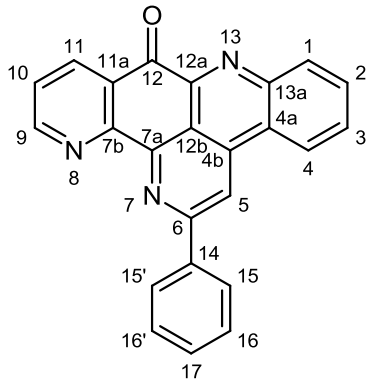
6-Methyl-ascididemin (**2.12**):



Prepared according to the general procedure using quinone **2.9** (111 mg, 0.41 mmol) and acetaldehyde (89.7 mg, 2.0 mmol). Reaction workup proceded utilising standard conditions. Purification was achieved by flash column chromatography (silica gel, 3.0 % methanol in dichloromethane) followed by precipitation from dichloromethane by addition of methanol and reduced pressure. This afforded **2.12** as an orange solid (47 mg, 39.1 %).

m.p. 281-283 ºC (decomp, lit.[68] 266-268 ºC); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.21 (1H, dd, *J* = 4.5, 1.8 Hz), 8.80 (1H, dd, *J* = 8.0, 1.7 Hz), 8.68 (1H, dd, *J* = 8.1, 1.2 Hz), 8.62 (1H, dd, *J* = 8.3, 1.3 Hz), 8.42 (1H, s), 8.00 (1H, ddd, *J* = 6.9, 6.9, 4 Hz), 7.92 (1H, ddd, *J* = 7.3, 6.9, 3 Hz), 7.66 (1H, dd, *J* = 7.9, 4.7 Hz), 3.08 (3H, s); EIMS *m/z* 297 $[M]^+$; HREIMS *m/z* 297.08988 (calcd for $C_{19}H_{11}N_3O$ 297.09021).

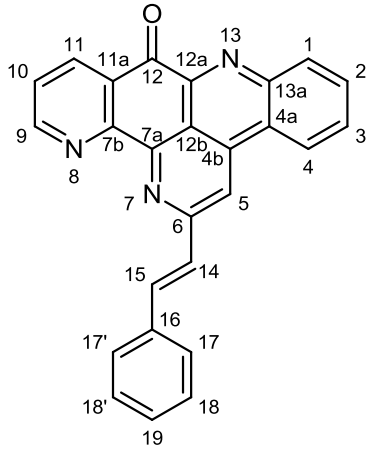
6-Phenyl-ascididemin (**2.13**):



Prepared according to the general procedure using quinone **2.9** (113 mg, 0.41 mmol) and benzaldehyde (215 mg, 2.02 mmol). Reaction workup was done utilising standard conditions. Purification was achieved by flash column chromatography (silica gel, 5.0 % methanol in dichloromethane) followed by precipitation from dichloromethane by addition of methanol and reduced pressure. This afforded **2.13** as a yellow solid (97 mg, 66 %).

m.p. 329-331 ºC (lit.[68] > 310 ºC); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.22 (1H, dd, *J* = 4.6, 1.7 Hz), 8.89 (1H, s), 8.80 (1H, dd, *J* = 7.9, 1.7 Hz), 8.77 (1H, dd, *J* = 8.0, 0.5 Hz), 8.62 (1H, dd, *J* = 8.2, 1.2 Hz), 8.36 (2H, d, *J* = 7.1 Hz), 8.00 (1H, ddd, *J* = 7.1, 7.0, 1.3 Hz), 7.93 (1H, ddd, *J* = 6.9, 6.9, 1.3 Hz), 7.67 (1H, dd, *J* = 7.9, 4.6 Hz), 7.59 (2H, t, *J* = 7.3 Hz), 7.53 (1H, t, *J* = 7.2 Hz); EIMS *m/z* 359 $[M]^+$; HREIMS *m/z* 359.10599 (calcd for $C_{24}H_{13}N_3O$ 359.10586).

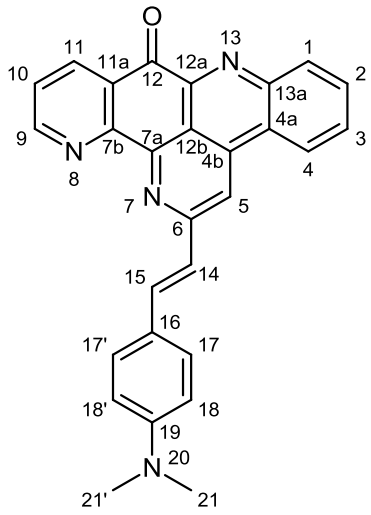
6-E-Styryl ascididemin (**2.14**):



Prepared according to the general procedure using quinone **2.9** (109 mg, 0.40 mmol) and cinnamaldehyde (268 mg, 2.0 mmol). Reaction workup was done utilising standard conditions. Purification was achieved by flash column chromatography (silica gel, 1.0 % methanol in dichloromethane) followed by precipitation from dichloromethane by addition of methanol and reduced pressure. This afforded **2.14** as a yellow solid (64 mg, 42 %).

m.p. 313-315 ºC (lit.[68] 281-283 ºC); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.21 (1H, dd, *J* = 4.6, 1.8, H-9), 8.78 (1H, dd, *J* = 7.9, 1.8, H-11), 8.69 (1H, dd, *J* = 8.1, 0.8 Hz, H-4), 8.58 (1H, dd, *J* = 8.2, 1.0 Hz, H-1), 8.56 (1H, s, H-5), 8.03 (1H, d, *J* = 16.1 Hz, H-15), 7.97 (1H, td, *J* = 7.6, 1.3 Hz, H-2), 7.90 (1H, td, *J* = 7.6, 1.3 Hz, H-3), 7.71 (2H, d, *J* = 7.3 Hz, H-17), 7.65 (1H, dd, *J* = 7.7, 4.5 Hz, H-10), 7.62 (1H, d, *J* = 15.9 Hz, H-14), 7.43 (2H, t, *J* = 7.4 Hz, H-18), 7.36 (1H, t, *J* = 7.3 Hz, H-19); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.0 (C-12), 156.3 (C-6), 155.5 (C-9), 152.4 (C-7b), 149.6 (C-7a), 146.0 (C-12a or C-13a), 145.9 (C-12a or C-13a), 138.7 (C-4b), 136.6 (C-11), 136.2 (C-15, C-16), 133.1 (C-1), 131.7 (C-2), 130.6 (C-3), 129.2 (C-11a), 129.1 (C-19), 128.9 (C-18), 127.8 (C-14), 127.6 (C-17), 125.5 (C-10), 123.6 (C-4a), 122.9 (C-4), 117.1 (C-12b), 113.5 (C-5); FABMS *m/z* 386 $[M+H]^+$; HRFABMS *m/z* 386.13000 (calcd for $C_{26}H_{16}N_3O$ 386.12934).

6-(4-Dimethylamino)-E-styryl-ascididemin (**2.25**):

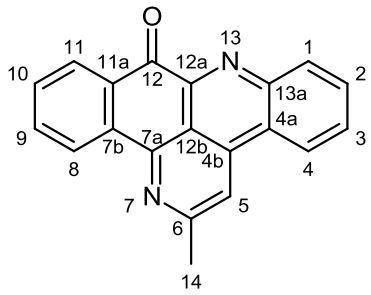


Prepared according to the general procedure using quinone **2.9** (109 mg, 0.40 mmol), 4-dimethylaminocinnamaldehyde (349 mg, 2.0 mmol) and using standard workup

conditions. Purification was achieved by flash column chromatography (silica gel, 1.5 % methanol in dichloromethane) followed by precipitation from dichloromethane by addition of hexane and reduced pressure. This afforded **2.25** as a green solid (66 mg, 42 %).

m.p. 267-268 ºC (decomp); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.19 (1H, dd, J = 4.6, 1.8 Hz, H-9), 8.73 (1H, dd, J = 7.9, 1.9 Hz, H-11), 8.61 (1H, dd, J = 7.9, 1.1 Hz, H-4), 8.51 (1H, dd, J = 8.2, 1.1 Hz, H-1), 8.41 (1H, s, H-5), 7.91 (1H, m, H-2), 7.86 (1H, d, J = 16.2 Hz, H-15), 7.85 (1H, m, H-3), 7.61 (1H, dd, J = 7.9, 4.6 Hz, H-10), 7.53 (2H, d, J = 8.8 Hz, H-17), 7.36 (1H, d, J = 16.1 Hz, H-14), 6.66 (2H, d, J = 8.8, Hz, H-18), 3.01 (6H, s, H-21); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 181.8 (C-12), 157.2 (C-6), 155.3 (C-9), 152.4 (C-7b), 150.8 (C-19), 149.2 (C-7a), 145.8 (C-12a), 145.8 (C-13a), 138.4 (C-4b), 136.5 (C-15), 136.5 (C-11), 132.9 (C-1), 131.4 (C-2), 130.2 (C-3), 129.1 (C-11a), 129.0 (C-17), 125.2 (C-10), 124.2 (C-16), 123.7 (C-4a), 123.1 (C-14), 122.8 (C-4), 116.6 (C-12b), 112.1 (C-5), 112.0 (C-18), 40.2 (C-21); FABMS *m/z* 429 $[M+H]^+$; HRFABMS *m/z* 429.17027 (calcd for $C_{28}H_{21}N_4O$ 429.17154).

6-Methyl-8-deaza-ascididemin (**2.28**):

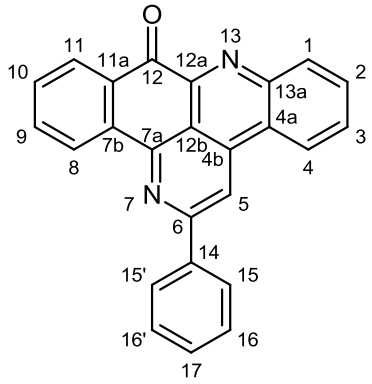


Prepared according to the general procedure using quinone **2.10** (107 mg, 0.39 mmol) and acetaldehyde (42 mg, 0.96 mmol). Reaction workup was done utilising standard conditions. Purification was achieved by flash column chromatography (silica gel, 5.0 % methanol in dichloromethane) followed by precipitation from dichloromethane by addition of methanol and reduced pressure. This afforded orange microcrystalline solid **2.28** (40 mg, 34.4 %).

m.p. 273-275 ºC (lit.[87] 271-273 ºC); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.83 (1H, dd, *J* = 7.9, 0.9 Hz, H-8), 8.54 (2H, dd, *J* = 8.5, 1.0 Hz, H-4, H-1), 8.44 (1H, dd, *J* = 7.8, 1.0

Hz, H-11), 8.15 (1H, s, H-5), 7.91 (1H, ddd, *J* = 7.1, 7.1, 1.4 Hz, H-2), 7.82 (1H, ddd, *J* = 7.1, 7.1, 1.2 Hz, H-3), 7.79 (H-1, td, *J* = 7.6, 1.5 Hz, H-9), 7.64 (1H, td, *J* = 7.5, 1.2 Hz, H-10), 2.87 (3H, s, H-14); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.4 (C-12), 158.7 (C-6), 149.8 (C-7a), 146.9 (C-12a), 145.8 (C-13a), 138.3 (C-4b), 136.2 (C-7b), 134.7 (C-9), 132.9 (C-1), 132.5 (C-11a), 131.2 (C-2), 130.9 (C-10), 129.9 (C-3), 128.6 (C-11), 125.7 (C-8), 123.4 (C-4a), 122.7 (C-4), 115.2 (C-12b), 113.9 (C-5), 25.5 (C-14); EIMS *m/z* 296 $[M]^+$; HREIMS *m/z* 296.09472 (calcd for $C_{20}H_{12}N_2O$ 296.09496); NMR data consistent with previous data, assignments based on previously recorded data.[87]

6-Phenyl-8-deaza-ascididemin (**2.29**):

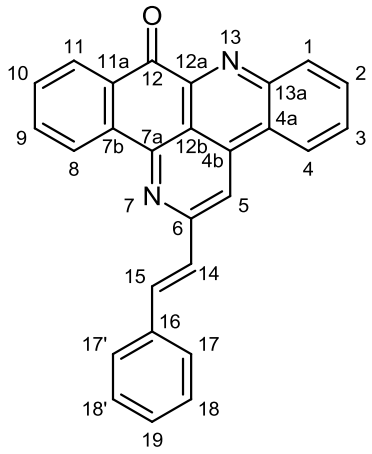


Prepared according to the general procedure using quinone **2.10** (111 mg, 0.41 mmol) and benzaldehyde (215 mg, 2.0 mmol). Reaction workup was done utilising standard conditions. Purification was achieved by flash column chromatography (silica gel, dichloromethane) followed by precipitation from dichloromethane by addition of methanol and reduced pressure. This afforded **2.29** as a yellow solid (53 mg, 36 %).

m.p. 306-308 ºC (lit.[87] 294-296 ºC); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.98 (1H, d, *J* = 7.8 Hz, H-8), 8.71 (1H, s, H-5), 8.66 (1H, dd, *J* = 7.6, 1.1 Hz, H-4), 8.56 (1H, dd, *J* = 8.2, 1.0 Hz, H-1), 8.44 (1H, dd, *J* = 7.8, 1.0 Hz, H-11), 8.32 (2H, dd, *J* = 8.1, 1.4 Hz, H-15), 7.94 (1H, td, *J* = 7.6, 1.4 Hz, H-2), 7.86 (1H, td, *J* = 8.2, 1.3 Hz, H-3), 7.80 (1H, td, *J* = 7.7, 1.3 Hz, H-9), 7.66 (1H, td, *J* = 7.5, 1.2 Hz, H-10), 7.61-7.50 (3H, m, H-16, H-17); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.3 (C-12), 156.3 (C-6), 150.1 (C-7a), 146.9 (C-12a), 146.0 (C-13a), 138.7 (C-4b), 138.6 (C-14), 136.2 (C-7b), 134.7 (C-9), 133.0 (C-1), 132.6 (C-11a), 131.4 (C-2), 131.1 (C-10), 130.1 (C-3), 130.0 (C-17),

129.0 (C-16), 128.6 (C-11), 127.5 (C-15), 125.8 (C-8), 123.8 (C-4a), 122.7 (C-4), 115.9 (C-12b), 110.9 (C-5); EIMS *m/z* 358 $[M]^+$; HREIMS *m/z* 358.11026 (calcd for $C_{25}H_{14}N_2O$ 358.11061); NMR data consistent with previous data, assignments based on previously recorded data.[87]

6-E-Styryl-8-deaza-ascididemin (**2.30**):

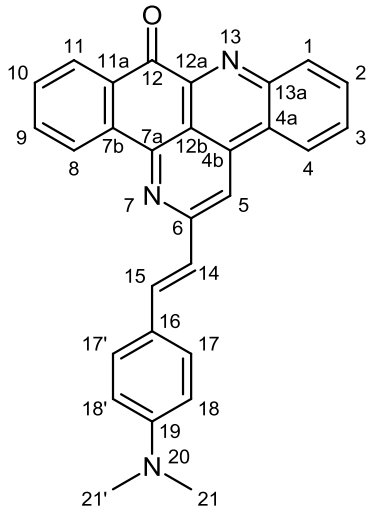


Prepared according to the general procedure using quinone **2.10** (111 mg, 0.41 mmol) and cinnamaldehyde (268 mg, 2.0 mmol). Reaction workup was done utilising standard conditions. Purification was achieved by flash column chromatography (silica gel, 5.0 % methanol in dichloromethane). This afforded **2.30** as a yellow solid (66 mg, 42 %).

m.p. 296-298 ºC (lit.[87] 303-305 ºC); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.84 (1H, dd, *J* = 7.7, 0.5 Hz, H-8), 8.45-48 (2H, m, *J* = H-1, H-4), 8.35 (1H, dd, *J* = 7.2, 1.2 Hz, H-11), 8.06 (1H, s, *J* = H-5), 7.96 (1H, d, *J* = 15.7 Hz, H-15), 7.85 (1H, td, *J* = 7.6, 1.1 Hz, H-2), 7.78 (1H, td, *J* = 7.8, 1.2 Hz, H-3), 7.76 (1H, td, *J* = 7.7, 0.9 Hz, H-9), 7.64 (2H, d, *J* = 7.3 Hz, H-17), 7.59 (1H, td, *J* = 7.5, 0.8 Hz, H-10), 7.44 (2H, t, *J* = 7.4 Hz, H-18), 7.38 (1H, t, *J* = 7.2 Hz, H-19), 7.24 (1H, d, *J* = 15.8 Hz, H-14); ^{13}C NMR ($CDCl_3$, 100 MHz) 182.2 (C-12), 154.4 (C-6), 149.9 (C-7a), 146.7 (C-12a), 146.0 (C-13a), 138.5 (C-4b), 136.3 (C-16), 136.0 (C-7b), 135.4 (C-15), 134.6 (C-9), 132.8 (C-1), 132.5 (C-11a), 131.3 (C-2), 131.0 (C-10), 129.9 (C-19), 128.9 (C-3), 128.8 (C-18), 128.5 (C-11), 127.5 (C-17), 127.1 (C-14), 125.7 (C-8), 123.5 (C-4a), 122.6 (C-4), 115.9 (C-12b), 113.2 (C-5); FABMS *m/z* 385 $[M+H]^+$; HRFABMS *m/z* 385.13423

(calcd for $C_{27}H_{17}N_2O$ 385.13409); NMR data consistent with previous data, assignments based on previously recorded data.[87]

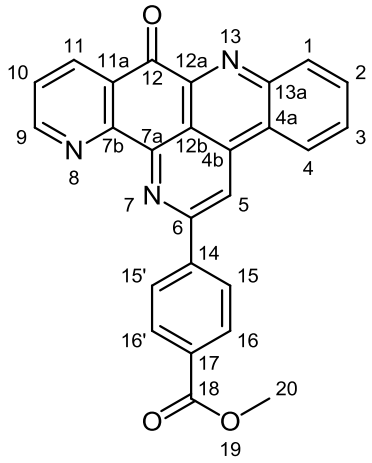
6-(4-Dimethylamino)-E-styryl-8-deaza-ascididemin (**2.31**):



Prepared according to the general procedure using quinone **2.10** (110 mg, 0.40 mmol), 4-dimethylaminocinnamaldehyde (347 mg, 1.98 mmol) and using standard workup conditions. Purification was achieved by flash column chromatography (silica gel, 1.0 % methanol in dichloromethane) followed by precipitation from dichloromethane by addition of hexane and reduced pressure. This afforded **2.31** as a purple solid (82 mg, 43 %).

m.p. >360 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.99 (1H, dd, *J* = 8.0, 0.8 Hz, H-8), 8.56 (1H, dd, *J* = 8.2, 1.0 Hz, H-4), 8.52 (1H, dd, *J* = 8.2, 1.0 Hz, H-1), 8.44 (1H, dd, *J* = 7.8, 1.1 Hz, H-11), 8.13 (1H, s, H-5), 8.03 (1H, d, *J* = 15.6 Hz, H-15), 7.89 (1H, ddd, *J* = 8.2, 7.1, 1.3 Hz, H-2), 7.84 (1H, td, *J* = 7.4, 1.4 Hz, H-9), 7.81 (1H, ddd, *J* = 7.7, 7.1, 1.4 Hz, H-3), 7.65 (1H, td, *J* = 7.6, 1.2 Hz, H-10), 7.57 (2H, d, *J* = 8.8 Hz, H-17), 7.17 (1H, d, *J* = 15.6 Hz, H-14), 6.74 (2H, d, *J* = 8.8 Hz, H-18), 3.04 (6H, s, H-21); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.6 (C-12), 155.7 (C-6), 150.9 (C-19), 149.9 (C-7a), 146.9 (C-12a), 146.1 (C-13a), 138.7 (C-4b), 136.4 (C-7b), 136.0 (C-15), 134.6 (C-9), 132.9 (C-1), 132.6 (C-11a), 131.2 (C-2), 130.9 (C-10), 129.7 (C-3), 128.9 (C-17), 128.5 (C-11), 125.8 (C-8), 124.4 (C-16), 123.8 (C-4a), 122.7 (C-4, 14*), 122.7 (C-4, 14*), 115.7 (C-12b), 112.1 (C-5, 18), 40.3 (C-21); EIMS *m/z* 427 $[M+H]^+$; HREIMS *m/z* 427.16758 (calcd for $C_{29}H_{21}N_3O$ 427.16846).

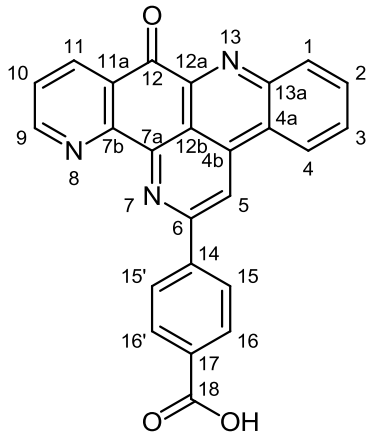
6-(4-(Methoxycarbonyl)phenyl)-ascididemin (**2.32**):



A mixture of tetracycle (**2.9**) (112 mg, 0.41 mmol), ammonium chloride (639 mg, 12 mmol) and methyl 4-formylbenzoate (337 mg, 2.1 mmol) in glacial acetic acid (50 mL) was refluxed under nitrogen for 1 hr. The reaction mixture was cooled to room temperature, diluted with water (200 mL) and neutralised with sodium bicarbonate. The mixture was extracted with dichloromethane (6 x 100 mL) and the combined organic layer was washed with water (2 x 200 mL). The organic layer was filtered through cotton wool and dried in vacuo. The resulting solid was washed with water and dried in vacuo. The solid was slurried with dichloromethane, filtered and washed a few times with dichloromethane. This afforded **2.32** as a yellow solid (116 mg, 69%).

Not sufficiently soluble to be fully characterised by NMR; m.p. > 360 ºC; IR (smear) ν_{max} 3074, 3042, 2999, 2950, 1714, 1684, 1600, 1573, 1283, 1114, 946, 768, 741 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.25 (1H, dd, J = 4.6, 1.8 Hz), 9.00 (1H, s), 8.84 (2H, m), 8.67 (1H, dd, J = 8.2, 1.0 Hz), 8.47 (2H, d, J = 8.5 Hz), 8.28 (2H, d, J = 8.4 Hz), 8.05 (1H, ddd, J = 8.1, 7.1, 1.3 Hz), 7.99 (1H, td, J = 8.1, 1.4 Hz), 7.71 (1H, dd, J = 7.8, 4.6 Hz), 3.99 (3H, s); no ms detected.

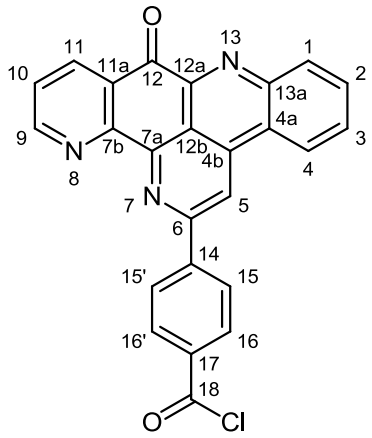
6-(4-Carboxyphenyl)-ascididemin (**2.33**):



To methyl ester **2.32** was added methanolic potassium hydroxide (1M, 5 mL) and the suspension was refluxed for 18 hours. The mixture was allowed to cool to room temperature and dried in vacuo. To the solid was added methanolic hydrochloric acid (1M, 10 mL) and the mixture dried in vacuo. The solid was washed with water (2 x 5 mL) and further dried in vacuo. The resulting yellow solid **2.33** was used without purification in following reactions.

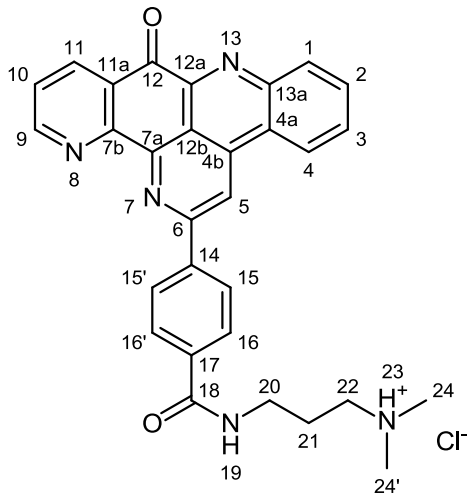
^{1}H NMR ($(CD_3)_2SO$, 600 MHz) δ 9.61 (1H, s, H-5), 9.37 (1H, d, *J* = 8.0 Hz, H-4), 9.24 (1H, dd, *J* = 4.5, 1.7 Hz, H-9), 8.79 (2H, d, *J* = 8.5 Hz, H-15), 8.71 (1H, dd, *J* = 7.8, 1.8 Hz, H-11), 8.50 (1H, dd, *J* = 8.2, 1.2 Hz, H-1), 8.22 (2H, d, *J* = 8.6 Hz, H-16), 8.13 (1H, td, *J* = 7.5, 1.4 Hz, H-2), 8.09 (1H, td, *J* = 7.5, 1.4 Hz, H-3), 7.87 (1H, dd, *J* = 7.9, 4.6 Hz, H-10).

6-(4-(Chlorocarbonyl)-phenyl)-ascididemin (**2.34**):



To acid **2.33** was added thionyl chloride (5 mL). The mixture was refluxed under nitrogen for 2 hrs and cooled to room temperature. Excess thionyl chloride was removed in vacuo, and the crude acid chloride **2.34** was used without purification in further reactions.

6-(4-(3-(Dimethylamino)propylcarbamoyl)phenyl)-ascididemin hydrochloride salt (**2.35**):

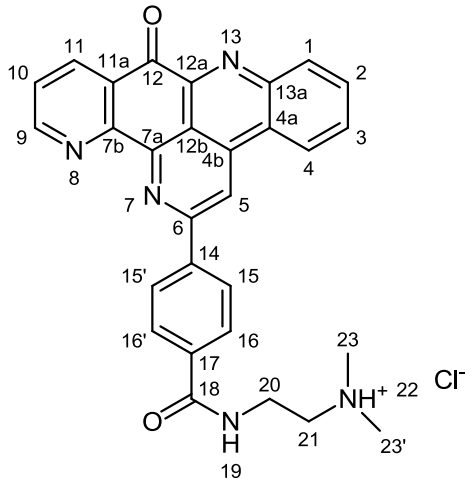


To acid chloride **2.34** (0.050 mmol) was added 3-(dimethylamino)propylamine (32 μL, 0.25 mmol) dissolved in dry dichloromethane (5mL) and stirred for 1 hr. The reaction mixture was diluted with dichloromethane (20 mL), and washed with water (2 x 50 mL). The organic layer was then extracted with aqueous HCl (0.1%, 4 x 25 mL). The combined acidic aqueous extractions were washed with dicloromethane (2 x 100 mL). The aqueous layer was dried in vacuo, dissolved in water and purified by reverse-phase C18 flash chromatography (50% methanol:water /0.1% HCl). The major yellow band was further purified via repeated LH20 column chromatography (methanol/ 0.05% TFA) and finally by reverse-phase C18 flash chromatography (50% methanol:water /0.1% HCl). This afforded **2.35** as a yellow to red solid (6.9 mg, 27% from **2.32**).

m.p. 227-229°C (decomp); IR (smear) ν_{max} 3065, 2963, 2700, 1676, 1655, 1639, 1631, 1598, 1577, 1546, 1511, 1501, 1476, 1467, 1460, 1441, 1412, 1386, 1302, 1276, 1160, 1089, 948, 861, 812, 778, 738 cm^{-1}; ^1H NMR ($(CD_3)_2SO$, 400 MHz) δ 10.10 (1H, br, H-23), 9.48 (1H, s, H-5), 9.28 (1H, d, *J* = 8.1 Hz, H-4), 9.19 (1H, dd, *J*

= 4.4, 1.9 Hz, H-9), 8.91 (1H, br t, *J* = 5.5 Hz, H-19), 8.71 (2H, d, *J* =8.4 Hz, H-15 and H-15'), 8.65 (1H, dd, *J* = 7.9, 1.8 Hz, H-11), 8.42 (1H, d, *J* = 8.0 Hz, H-1), 8.13 (2H, d, *J* = 8.4 Hz, H-16 and H-16'), 8.08 (1H, t, *J* = 7.4 Hz, H-2), 8.03 (1H, t, *J* = 7.5 Hz, H-3), 7.84 (1H, dd, *J* = 7.8, 4.6 Hz, H-10), 3.42 (2H, q, *J* = 5.9 Hz, H-20), 3.15 (2H, q, *J* = 6.9 Hz, H-22), 2.80 (6H, d, *J* = 4.8 Hz, H-24 and H-24), 1.99 (2H, p, *J* = 7.0 Hz, H-21); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz) δ 181.0 (C-12), 166.0 (C-18), 154.5 (C-9), 154.4 (C-6), 151.4 (C-7b), 148.9 (C-7a*), 146.1 (C-12a*), 145.1 (C-13a), 140.2 (C-14), 138.4 (C-4b), 135.9 (C-11), 135.2 (C-17), 132.0 (C-2), 131.8 (C-1), 130.5 (C-3), 129.2 (C-11a), 127.7 (C-16, C-16'), 127.4 (C-15, C-15'), 125.9 (C-10), 124.6 (C-4), 123.4 (C-4a), 117.3 (C-12b), 113.5 (C-5), 54.6 (C-22), 42.1 (C-24, C-24'), 36.4 (C-20), 24.3(C-21); FABMS *m/z* 488 $[M+H]^+$; HRFABMS *m/z* 488.20845 (calcd for $C_{30}H_{26}N_5O_2$ 488.20865); purity 98.4% t_R = 7.48 min.

6-(4-(3-(Dimethylamino)ethylcarbamoyl)phenyl)-ascididemin hydrochloride salt (**2.36**):

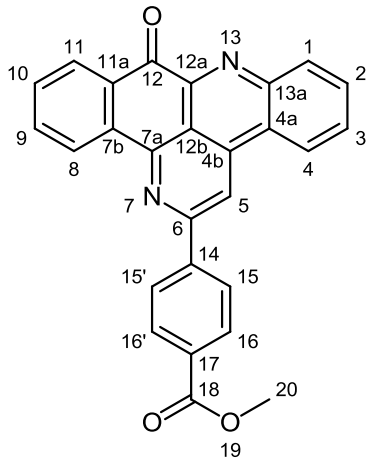


To acid chloride **2.34** (0.046 mmol) was added N,N-dimethylaminoethylenediamine (25 μL, 0.23 mmol) dissolved with dry dichloromethane (5 mL) and stirred for 1 hr. The reaction mixture was diluted in dichloromethane (20 mL), and washed with water (2 x 25 mL). The organic layer was then extracted with aqueous HCl (0.1%, 4 x 20 mL). The combined acidic aqueous extractions were washed with dicloromethane (2 x 100 mL). The aqueous layer was dried in vacuo, dissolved in water and purified by reverse-phase C18 flash chromatography (50% methanol:water /0.1% HCl). The major yellow band was further purified via repeated LH20 column chromatography

(methanol/ 0.05% TFA) and finally by reverse-phase C18 flash chromatography (50% methanol:water /0.1% HCl). This afforded **2.36** as a yellow to red solid (12.1 mg, 52% from **2.32**).

m.p. 284-286°C (decomp); IR (smear) ν_{max} 3050, 2960, 2924, 2853 2691, 1694, 1660, 1650, 1644, 1592, 1547, 1513, 1462, 1442, 1417, 1387, 1376, 1298, 1238, 1159, 1040, 1010, 988, 948, 870, 839, 814, 779, 738 cm^{-1}; ^1H NMR ($(CD_3)_2SO$, 400 MHz) δ 10.22 (1H, br, H-22), 9.54 (1H, s, H-5), 9.33 (1H, dd, *J* = 8.1, 1.0 Hz, H-4), 9.22 (1H, dd, *J* = 4.7, 1.7 Hz, H-9), 9.08 (1H, br t, *J* = 5.6 Hz, H-19), 8.77 (2H, d, *J* = 8.4 Hz, H-15 and H-15'), 8.69 (1H, dd, *J* = 7.8, 1.8 Hz, H-11), 8.45 (1H, dd, *J* = 8.1, 1.1 Hz, H-1), 8.21 (2H, d, *J* = 8.4 Hz, H-16 and H-16'), 8.10 (1H, tt, *J* = 7.4, 1.2 Hz, H-2), 8.05 (1H, tt, *J* = 7.5, 1.1 Hz, H-3), 7.87 (1H, dd, *J* = 7.9, 4.6 Hz, H-10), 3.72 (2H, q, *J* = 5.8 Hz, H-20), 3.34 (2H, q, *J* =5.8 Hz, H-21), 2.88(6H, d, *J* = 4.9 Hz, H-23 and H-23'); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz) δ 181.1 (C-12), 166.2 (C-18), 154.5 (C-6), 154.3 (C-9), 151.5 (C-7b), 149.0 (C-7a), 146.2 (C-12a), 145.1 (C-13a), 140.5 (C-14), 138.5 (C-4b), 136.0 (C-11), 134.8 (C-17), 132.0 (C-2), 131.8 (C-1), 130.5 (C-3), 129.3 (C-11a), 128.0 (C-16, C-16'), 127.4 (C-15, C-15'), 125.9 (C-10), 124.6 (C-4), 123.5 (C-4a), 117.4 (C-12b), 113.6 (C-5), 56.0 (C-21), 42.4 (C-23, C-23'), 34.6(C-20); FABMS *m/z* 474 $[M+H]^+$; HRFABMS *m/z* 474.19328 (calcd for $C_{29}H_{24}N_5O_2$ 474.19300); purity 98.0% t_R = 7.40 min.

6-(4-(Methoxycarbonyl)phenyl)-8-deaza-ascididemin (**2.37**):

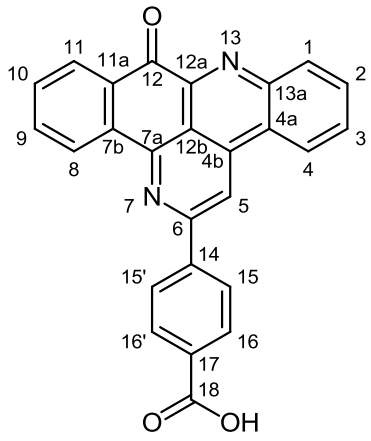


A mixture of tetracycle **2.10** (111 mg, 0.41 mmol), ammonium chloride (656 mg, 12 mmol) and methyl 4-formylbenzoate (322 mg, 2.0 mmol) in glacial acetic acid (50

mL) was refluxed under nitrogen for 1 hr. The reaction mixture was cooled to room temperature, diluted with water (200 mL) and neutralised with sodium bicarbonate. The mixture was extracted with dichloromethane (6 x 100 mL) and the combined organic layer was washed with water (2 x 200 mL). The organic layer was filtered through cotton wool and dried in vacuo. The resulting solid was washed with water and dried in vacuo. The solid was slurried with dichloromethane, filtered and washed a few times with dichloromethane. This afforded **2.37** as a yellow solid (111 mg, 65%).

Not sufficiently soluble to be fully characterised by NMR; m.p. 347-348 ºC; IR (smear) ν_{max} 3092, 3073, 3010, 2959, 1714, 1686, 1606, 1592, 1574, 1432, 1290, 1267, 1114, 778, 770, 736; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.06 (1H, d, *J* = 7.8 Hz), 8.87 (1H, s), 8.76 (1H, d, *J* = 8.1 Hz), 8.63 (1H, d, *J* = 8.1 Hz), 8.51 (1H, d, *J* = 7.7 Hz), 8.46 (2H, d, *J* = 8.2 Hz), 8.27 (2H, d, *J* = 8.3 Hz), 8.00 (1H, t, *J* = 7.6 Hz), 7.93 (1H, t, *J* = 7.5 Hz), 7.89 (1H, t, *J* = 7.6 Hz), 7.72 (1H, t, *J* = 7.6 Hz), 4.00 (3H, s); no ms detected.

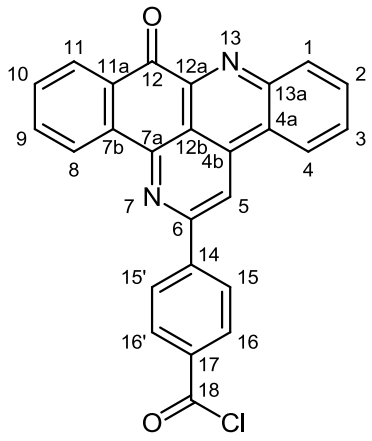
6-(4-Carboxyphenyl)-8-deaza-ascididemin (**2.38**):



To methyl ester **2.37** was added methanolic potassium hydroxide (1M, 5 mL) and the suspension was refluxed for 18 hours. The mixture was allowed to cool to room temperature and dried in vacuo. To the solid was added methanolic hydrochloric acid (1M, 10 mL) and the mixture dried in vacuo. The solid was washed with water (2 x 5 mL) and further dried in vacuo. The resulting yellow solid **2.38** was used without purification in following reactions.

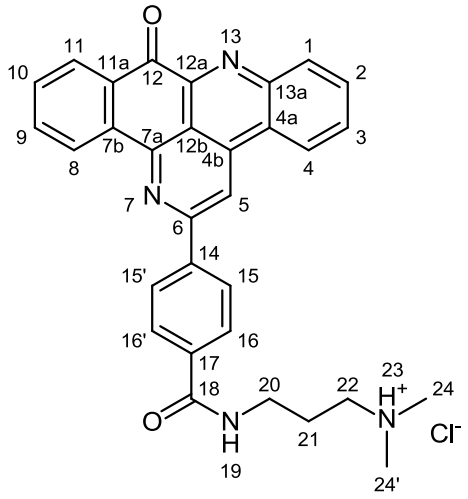
^{1}H NMR ($(CD_3)_2SO$, 600 MHz) δ 9.27 (1H, s, H-5), 9.14 (1H, d, *J* = 7.7 Hz, H-4), 8.85 (1H, d, *J* = 7.8 Hz, H-8), 8.62 (2H, d, *J* = 8.3 Hz, H-15 and H-15'), 8.34 (1H, dd, *J* = 8.1, 0.8 Hz, H-1), 8.21 (1H, dd, *J* = 7.7, 1.0 Hz, H-11), 8.12 (2H, d, *J* = 8.3 Hz, H-16 and H-16'), 8.02 (1H, td, *J* = 7.5, 1.1 Hz, H-2), 7.95 (1H, td, *J* = 7.5, 1.0 Hz, H-3), 7.91 (1H, td, *J* = 7.5, 1.2 Hz, H-9), 7.73(1H, td, *J* = 7.4, 1.0 Hz, H-10); ^{13}C NMR ($(CD_3)_2SO$, 150 MHz) δ 181.0 (C-12), 167.0 (C-18), 153.6 (C-6), 149.2 (C-7a), 146.5 (C-12a), 145.3 (C-13a), 141.6 (C-14), 138.4 (C-4b), 135.4(C-7b), 134.8 (C-9), 132.3 (C-11a), 131.8 (C-2), 131.7 (C-1, C-17), 131.2 (C-10), 130.2 (C-3), 129.7 (C-3), 127.7 (C-16, C-16'), 127.4 (C-15, C-15'), 125.3 (C-8), 124.4 (C-4), 123.4 (C-4a), 115.9 (C-12b), 112.7 (C-5).

6-(4-(Chlorocarbonyl)-phenyl)-8-deaza-ascididemin (**2.39**):



To acid **2.38** was added thionyl chloride (5 mL). The mixture was refluxed under nitrogen for 2 hrs and cooled to room temperature. Excess thionyl chloride was removed in vacuo, and the crude acid chloride **2.39** was used without purification in further reactions.

6-(4-(3-(Dimethylamino)propylcarbamoyl)phenyl)-8-deaza-ascididemin hydrochloride salt (**2.40**):

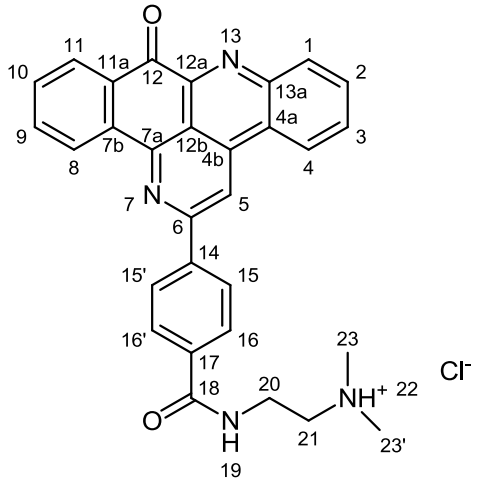


To acid chloride **2.39** (0.051 mmol) was added 3-(dimethylamino)propylamine (32 μL, 0.25 mmol) dissolved in dry dichloromethane (5mL) and stirred for 1 hr. The reaction mixture was diluted with dichloromethane (20 mL), and washed with water (2 x 50 mL). The organic layer was then extracted with aqueous HCl (0.1%, 4 x 25 mL). The combined acidic aqueous extractions were washed with dicloromethane (2 x 100 mL). The aqueous layer was dried in vacuo, dissolved in water and purified by reverse-phase C18 flash chromatography (60% methanol:water /0.1% HCl). The major yellow band was further purified via repeated LH20 column chromatography (methanol/ 0.05% TFA) and finally by reverse-phase C18 flash chromatography (60% methanol:water /0.1% HCl). This afforded **2.40** as a yellow to red solid (13.3 mg, 50 % from **2.37**).

m.p. 235-236°C (decomp); IR (smear) ν_{max} 3069, 2960, 2852, 2714, 1604, 1656, 1604, 1591, 1571, 1545, 1512, 1500, 1474, 1460, 1434, 1413, 1388, 1348, 1299, 1269, 1245, 1162, 1120, 1088, 1054, 1031, 1012, 948, 854, 776, 766, 737 cm^{-1}; ^1H NMR ($(CD_3)_2SO$, 400 MHz) δ 10.62 (1H, br, H-23), 9.26 (1H, s, H-5), 9.16 (1H, d, *J* = 8.0 Hz, H-4), 8.97 (1H, br t, *J* = 5.6 Hz, H-19), 8.85 (1H, d, *J* = 7.7 Hz, H-8), 8.61 (2H, d, *J* = 8.2 Hz, H-15 and H-15'), 8.33 (1H, d, *J* = 8.0 Hz, H-1), 8.20 (1H, d, *J* = 7.6 Hz, H-11), 8.11 (2H, d, *J* = 8.2 Hz, H-16 and H-16'), 8.02 (1H, t, *J* = 7.5 Hz, H-2), 7.95 (1H, t, *J* = 7.6 Hz, H-3), 7.91 (1H, t, *J* = 7.5 Hz, H-9), 7.73 (1H, t, *J* = 7.3 Hz, H-10), 3.43 (2H, q, *J* = 6.2 Hz, H-20), 3.16 (2H, q, *J* = 7.0 Hz, H-22), 2.78 (6H, d, *J* =

4.9 Hz, H-24 and H-24'), 2.02 (2H, p, J = 7.2 Hz, H-21); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz) δ 180.9 (C-12), 165.8 (C-18), 153.8 (C-6), 149.1 (C-7a), 146.4 (C-12a), 145.2 (C-13a), 140.1 (C-14), 138.4 (C-4b), 135.4 (C-7b), 135.1 (C-17), 134.7 (C-9), 132.2 (C-11a), 131.8 (C-2), 131.6 (C-1), 131.2 (C-10), 130.1 (C-3), 127.7 (C-16, C-16'), 127.6 (C-11), 127.2 (C-15, C-15'), 125.3 (C-8), 124.5 (C-4), 123.3 (C-4a), 115.8 (C-12b), 112.3 (C-5), 54.4 (C-22), 41.9 (C-24, C-24'), 36.4 (C-20), 24.2 (C-21); FABMS *m/z* 487 $[M+H]^+$; HRFABMS *m/z* 487.21380 (calcd for $C_{31}H_{27}N_4O_2$ 487.21340); purity 98.4% t_R = 7.15 min.

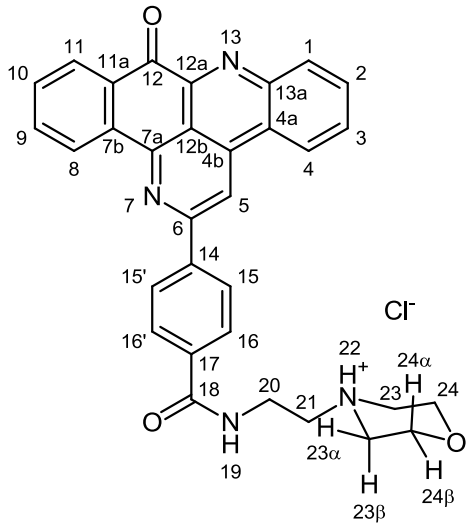
6-(4-(3-(Dimethylamino)ethylcarbamoyl)phenyl)-8-deaza-ascididemin hydrochloride salt (**2.41**):



To acid chloride **2.39** (0.057 mmol) was added N,N-dimethylaminoethylenediamine (31 μL, 0.28 mmol) dissolved in dry dichloromethane (5mL) and stirred for 1 hr. The reaction mixture was diluted with dichloromethane (20 mL), and washed with water (2 x 50 mL). The organic layer was then extracted with aqueous HCl (0.1%, 4 x 25 mL). The combined acidic aqueous extractions were washed with dicloromethane (2 x 100 mL). The aqueous layer was dried in vacuo, dissolved in water and purified by reverse-phase C18 flash chromatography (60% methanol:water /0.1% HCl). The major yellow band was further purified via repeated LH20 column chromatography (methanol/ 0.05% TFA) and finally by reverse-phase C18 flash chromatography (60% methanol:water /0.1% HCl). This afforded **2.41** as a yellow to red solid (10.2 mg, 35% from **2.37**).

m.p. 283-284°C (decomp); IR (smear) ν_{max} 3070, 2967, 2926, 2858, 2694, 1655, 1648, 1642, 1638, 1591, 1572, 1561, 1543, 1536, 1509, 1500, 1475, 1468, 1459, 1432, 1413, 1389, 1376, 1348, 1302, 1269, 1160, 1102, 1054, 1031, 1013, 986, 948, 854, 776, 768, 736 cm^{-1}; ^1H NMR ($(CD_3)_2SO$, 400 MHz) δ 10.65 (1H, br, H-22), 9.28 (1H, s, H-5), 9.17 (1H, dd, *J* = 8.2, 0.7 Hz, H-4), 9.16 (1H, br, H-19), 8.81 (1H, dd, *J* = 7.8, 1,6 Hz, H-8), 8.63 (2H, d, *J* = 8.5 Hz, H-15 and H-15'), 8.33 (1H, dd, *J* =8.2, 1.1 Hz, H-1), 8.20 (1H, dd, *J* = 7.6, 1.2 Hz, H-11), 8.18 (2H, d, *J* = 8.6 Hz, H-16 and H-16'), 8.02 (1H, td, *J* = 7.6, 1.2 Hz, H-2), 7.95 (1H, td, *J* = 7.6, 1.3 Hz, H-3), 7.91 (1H, td, *J* = 7.7, 1.3 Hz, H-9), 7.73 (1H, td, *J* = 7.5, 1.1 Hz, H-10), 3.74 (2H, q, *J* = 5.8 Hz, H - 20), 3.35 (2H, q, *J* = 5.7 Hz, H-21), 2.88 (6H, d, *J* = 4.9 Hz, H-23 and H-23'); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz) δ 180.9 (C-12), 166.1 (C-18), 153.7 (C-6), 149.1 (C-7a), 146.4 (C-12a), 145.2 (C-13a), 140.2 (C-14), 138.4 (C-4b), 135.4 (C-7b), 134.8 (C-9*), 134.7 (C-17*), 132.2 (C-11a), 131.8 (C-2), 131.6 (C-1), 131.2 (C-10), 130.2 (C-3), 128.0 (C-16, C-16'), 127.6 (C-11), 127.1 (C-15, C-15'), 125.3 (C-8), 124.5 (C-4), 123.4 (C-4a), 115.8 (C-12b), 112.4 (C-5), 56.0 (C-21), 42.3 (C-23, C-23'), 34.6 (C-20); FABMS *m/z* 473 $[M+H]^+$; HRFABMS *m/z* 473.19775 (calcd for $C_{30}H_{25}N_4O_2$ 473.19775); purity 96.2% t_R = 7.23 min.

6-(4-[[[2-(4-Morpholinyl)ethyl]amino]carbonyl]-phenyl)-8-deaza-ascididemin hydrochloride salt (**2.42**):

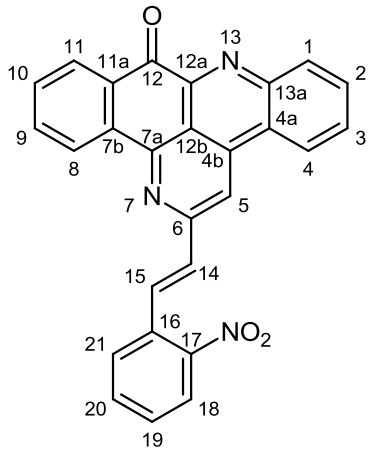


To acid chloride **2.39** (0.073 mmol) was added 2-(4-Morpholino)ethylamine (50 μL, 0.38 mmol) dissolved in dry dichloromethane (5 mL) and stirred for 1 hr. The reaction

mixture was diluted with dichloromethane (20 mL), and washed with water (2 x 25 mL). The organic layer was then extracted with aqueous HCl (0.1%, 4 x 25 mL). The combined acidic aqueous extractions were washed with dicloromethane (2 x 50 mL). The aqueous layer was dried in vacuo, dissolved in water and purified by reverse-phase C18 flash chromatography (70% methanol:water /0.1% HCl). The major yellow band was further purified by reverse-phase C18 flash chromatography (60% methanol:water /0.1% HCl). This afforded **2.42** as a yellow to red solid (9.23 mg, 23% from **2.37**).

m.p. 200-201 ºC (decomp.); IR (smear) ν_{max} 3360, 3080, 1651, 1604, 1589, 1569, 1539, 1410, 1294, 1268, 1099, 853, 765, 735 cm^{-1}; ^{1}H NMR ($(CD_3)_2SO$, 400 MHz) δ 11.35 (1H, br, H-22), 9.20 (2H, s, H-19, H-5), 9.11 (1H, d, *J* = 7.7 Hz, H-4), 8.78 (1H, d, *J* = 7.7 Hz, H-8), 8.57 (2H, d, *J* = 8.5 Hz, H-15, H-15'), 8.28 (1H, d, *J* = 8.1 Hz, H-1), 8.16 (2H, d, *J* = 8.5 Hz, H-16, H-16'), 8.15 (1H, dd, *J* = 8.8, 0.9 Hz, H-11), 7.98 (1H, td, *J* = 7.5, 1.1 Hz, H-2), 7.91 (1H, td, *J* = 7.7, 0.8 Hz, H-3), 7.87 (1H, td, *J* = 7.6, 1.3 Hz, H-9), 7.69 (1H, td, *J* = 7.5, 1.0 Hz, H-10), 4.01 (2H, dd, *J* = 12.4, 2.8 Hz, H-24β), 3.92 (2H, t, *J* = 11.4 Hz, H-24α), 3.81 (2H, q, *J* = 5.7 Hz, H-20), 3.62 (2H, d, *J* = 12.1 Hz, H-23α), 3.40 (2H, q, *J* = 5.5 Hz, H-21), 3.19 (2H, m, H-23β); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz) δ 180.8 (C-12), 165.9 (C-18), 153.6 (C-6), 149.0 (C-7a), 146.3 (C-12a), 145.1 (C-13a), 140.2 (C-14), 138.3 (C-4b), 135.3 (C-7b), 134.7 (C-9), 134.6 (C-17), 132.1 (C-11a), 131.7 (C-2), 131.6 (C-1), 131.1 (C-10), 130.1 (C-3), 127.9 (C-16, C-16'), 127.5 (C-11), 127.1 (C-15, C-15'), 125.2 (C-8), 124.4 (C-4), 123.3 (C-4a), 115.7 (C-12b), 112.3 (C-5), 63.1 (C-24, C-24'), 55.6 (C-21), 51.2 (C-23, C-23'), 33.7 (C-20); FABMS *m/z* 515 $[M+H]^+$; HRFABMS *m/z* 515.20898 (calcd for $C_{32}H_{27}N_4O_3$ 512.20832); purity 99.8% t_R = 6.91 min.

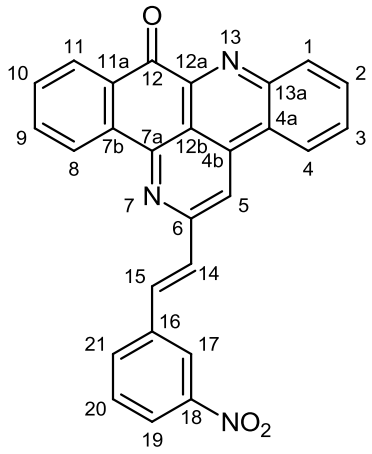
6-(2-Nitro-E-styryl)-8-deaza-ascdidemin (**2.43**):



Prepared according to the general procedure using quinone **2.10** (111 mg, 0.41 mmol) and 2-nitrocinnamaldehyde (353 mg, 2.0 mmol). Reaction workup used sodium bicarbonate workup, the product was purified by silica gel flash chromatography (1% methanol:dichloromethane) followed by extraction of the solid material with small volumes of dichloromethane. This afforded **2.43** as a yellow solid (89 mg, 51 %).

m.p. 275-276 ºC; IR ν_{max} 3420, 3071, 1673, 1602, 1588, 1572, 1516, 1401, 1345, 1271, 1053, 961, 948, 759, 742 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.01 (1H, dd, *J* = 7.9, 1.0 Hz, H-8), 8.66 (1H, dd, *J* = 8.2, 1.2 Hz, H-4), 8.63 (1H, d, *J* = 15.6 Hz, H-15), 8.61 (1H, dd, *J* = 8.2, 1.1 Hz, H-1), 8.50 (1H, dd, *J* = 7.8, 1.2 Hz, H-11), 8.40 (1H, s, H-5), 8.08 (1H, dd, *J* = 8.2, 1.3 Hz, H-18), 7.98 (1H, ddd, *J* = 8.3, 7.0, 1.4 Hz, H-2), 7.94 (1H, bd, *J* = 7.8 Hz, H-21), 7.91 (1H, m, H-9), 7.89 (1H, m, H-3), 7.72 (1H, m, H-20), 7.69 (1H, m, H-10), 7.54 (1H, td, *J* = 7.8, 1.3 Hz, H-19), 7.45 (1H, d, *J* = 15.6 Hz, H-14); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.2 (C-12), 153.7 (C-6), 150.4 (C-7a), 148.5 (C-17), 146.8 (C-12a), 146.1 (C-13a), 138.6 (C-4b), 135.9 (C-7b), 135.0 (C-9), 133.3 (C-20), 133.0 (C-1), 132.6 (C-11a), 132.2 (C-14, C-16), 131.5 (C-2), 131.3 (C-10), 130.4 (C-15), 130.1 (C-3), 129.1 (C-19), 128.6 (C-11), 128.5 (C-21), 125.9 (C-8), 125.0 (C-18), 123.5 (C-4a), 122.7 (C-4), 116.3 (C-12b), 114.1 (C-5); FABMS *m/z* 430 $[M+H]^+$; HRFABMS *m/z* 430.11908 (calcd for $C_{27}H_{16}N_3O_3$ 430.11917).

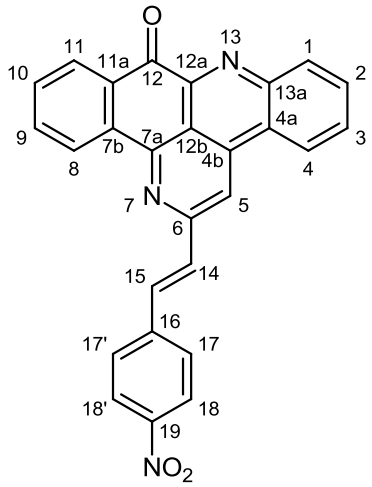
6-(3-Nitro-E-styryl)-8-deaza-ascdidemin (**2.44**):



Prepared according to the general procedure using quinone **2.10** (110 mg, 0.40 mmol) and 3-nitrocinnamaldehyde (350 mg, 2.0 mmol). Reaction workup was done utilising modified conditions. The cooled reaction mix was poured onto water and neutralised with sodium bicarbonate. The resulting suspension was filtered through cotton wool and the cotton wool washed extensively with water. The cotton wool was extensively extracted with dichloromethane and the combined extracts filtered through cotton wool, dried in vacuo to give **2.44** as a yellow solid (79 mg, 46 %).

Not sufficiently soluble to be fully characterised by NMR, m.p. > 360 ºC; IR ν_{max} 3452, 3076, 3034, 1682, 1589, 1570, 1405, 1349, 969, 768, 739, 676 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.08 (1H, d, *J* = 7.9 Hz), 8.69 (1H, d, *J* = 8.1 Hz), 8.63 (1H, d, *J* = 8.4 Hz), 8.60 (1H, s), 8.53 (1H, d, *J* = 7.3 Hz), 8.41 (1H, s), 8.26 (1H, d, *J* = 15.7 Hz), 8.22 (1H, d, *J* = 9.6 Hz), 8.01 (2H, m), 7.92 (2H, t, *J* = 7.5 Hz), 7.74 (1H, t, *J* = 7.6 Hz), 7.64 (1H, t, *J* = 7.9 Hz), 7.62 (1H, d, *J* = 15.8 Hz); FABMS *m/z* 430 $[M+H]^+$; HRFABMS *m/z* 430.12027 (calcd for $C_{27}H_{16}N_3O_3$ 430.11917).

6-(4-Nitro-E-styryl)-8-deaza-ascdidemin (**2.45**):



Prepared according to the general procedure using quinone **2.10** (109 mg, 0.40 mmol) and 4nitrocinnamaldehyde (355. mg, 2.0 mmol). Reaction workup was done utilising modified conditions. The cooled reaction mix was poured onto water and neutralised with sodium bicarbonate. The resulting suspension was filtered through cotton wool and the cotton wool washed extensively with water. The cotton wool was extensively extracted with dichloromethane and the combined extracts filtered through cotton wool, dried in vacuo to give **2.45** as a yellow solid (124 mg, 73 %).

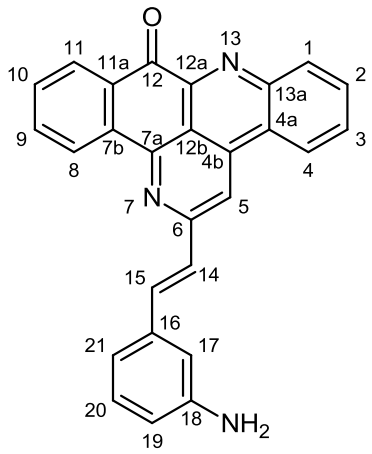
Not sufficiently soluble to be fully characterised by NMR, m.p. 319-320 ºC; IR ν_{max} 3481, 3065, 1679, 1590, 1573, 1506, 1333, 1269, 1105, 971, 948, 749 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.05 (1H, dd, *J* = 7.9, 0.9 Hz), 8.66 (1H, dd, *J* = 8.1, 1.0 Hz), 8.62 (1H, dd, *J* = 8.5, 1.1 Hz), 8.52 (1H, dd, *J* = 7.8, 1.2 Hz), 8.40 (1H, s), 8.32 (2H, d, *J* = 8.8 Hz), 8.24 (1H, d, *J* = 15.7 Hz), 7.99 (1H, ddd, *J* = 8.3, 7.1, 1.4 Hz), 7.91 (2H, m), 7.87 (2H, d, *J* = 8.8 Hz), 7.73 (1H, td, *J* = 7.8, 1.3 Hz), 7.60 (1H, d, *J* = 15.7 Hz); FABMS *m/z* 430 $[M+H]^+$; HRFABMS *m/z* 430.11894 (calcd for $C_{27}H_{16}N_3O_3$ 430.11917).

General reduction method used to prepare compounds **2.46**-**2.48**:

A mixture of the nitro compound (1 eq) and tin powder (10 eq) in conc. HCl (10 mL) was refluxed overnight. On cooling the mixture was poured onto ice (200 mL), neutralised with sodium hydrogen carbonate and made basic (pH > 10) with sodium

hydroxide. The aqueous material was extracted with dichloromethane (3 x 100 mL) and the combined organic layers were filtered through cotton wool and washed with water (2 x 100 mL). The organic layer was then dried in vacuo to yield crude material, which was a red to purple solid. The crude product was purified by reverse-phase C18 flash chromatography (80% methanol:water /0.1% HCl). Sodium hydroxide was added to a solution of purified material in methanol water until precipitation occurred. The mixture was reduced in volume in vacuo until the majority of methanol was removed. The aqueous material was extracted with dichloromethane (3 x 50 mL) and the combined organic layers were washed with water (2 x 50 mL). The organic was dried in vacuo to give product as the free base.

6-(3-Amino-E-styryl)-8-deaza-ascdidemin (**2.46**):

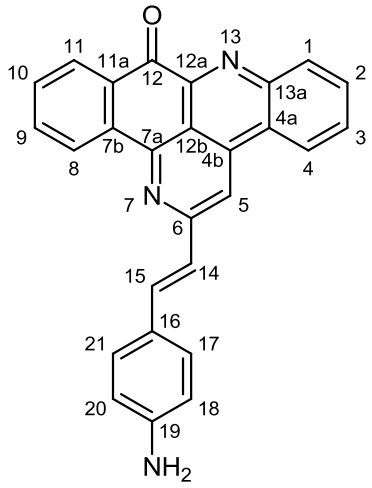


Prepared according to the general procedure using nitrostyryl **2.44** (43 mg, 0.10 mmol). Reaction workup was performed utilising standard conditions to give a red solid **2.46** (35 mg, 86 %).

Not sufficiently soluble to be fully characterised by NMR, m.p. 314-315 ºC (decomp.); IR ν_{max} 3468, 3327, 3211, 3055, 1678, 1629, 1597, 1588, 1566, 1457, 1400, 1313, 1270, 976, 768 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.06 (1H, dd, *J* = 8.1, 1.1 Hz), 8.66 (1H, dd, *J* = 7.9, 1.1 Hz), 8.60 (1H, dd, *J* = 8.2, 1.0 Hz), 8.50 (1H, dd, *J* = 7.9, 1.2 Hz), 8.32 (1H, s), 8.10 (1H, d, *J* = 15.9 Hz), 7.96 (1H, ddd, *J* = 8.1, 7.1, 1.4 Hz), 7.89 (2H, m), 7.70 (1H, td, *J* = 7.9, 1.4 Hz), 7.42 (1H, d, *J* = 15.8 Hz), 7.25 (1H, obsc), 7.15 (1H, bd, *J* = 7.5 Hz), 7.05 (1H, s), 6.72 (1H, dd, *J* = 7.9, 1.8 Hz), 3.77 (2H,

br); FABMS *m/z* 400 $[M+H]^+$; HRFABMS *m/z* 400.14503 (calcd for $C_{27}H_{18}N_3O$ 400.14499); purity 99.8% t_R = 6.56 min.

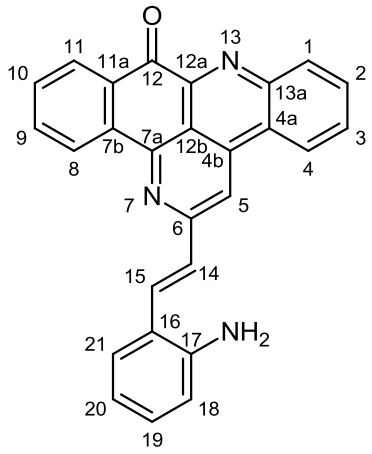
6-(4-Amino-E-styryl)-8-deaza-ascdidemin (**2.47**):



Prepared according to the general procedure using nitrostyryl **2.45** (47 mg, 0.11 mmol). Reaction workup was performed utilising standard conditions to give a purple solid **2.47** (43 mg, 97 %).

Not sufficiently soluble to be fully characterised by NMR, m.p. 325-326 ºC; IR ν_{max} 3452, 3346, 3210, 1678, 1620, 1627, 1581, 1562, 1546, 1516, 1400, 1300, 1271, 1171, 973, 765 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.05 (1H, dd, *J* = 7.8, 1.1 Hz), 8.64 (1H, dd, *J* = 8.3, 1.1 Hz), 8.57 (1H, dd, *J* = 8.2, 1.1 Hz), 8.49 (1H, dd, *J* = 7.8, 1.2 Hz), 8.24 (1H, s), 8.10 (1H, d, *J* = 15.7 Hz), 7.94 (1H, ddd, *J* = 8.1, 6.9, 1.3 Hz), 7.88 (1H, td, *J* = 7.3, 1.3 Hz), 7.86 (1H, td, *J* = 6.8, 1.2 Hz), 7.69 (1H, td, *J* = 7.5, 1.1 Hz), 7.55 (2H, d, *J* = 8.4 Hz), 7.27 (1H, d (half obsc), *J* = 16.9 Hz), 6.75 (2H, d, *J* = 8.4 Hz), 3.90 (2H, br); FABMS *m/z* 400 $[M+H]^+$; HRFABMS *m/z* 400.14488 (calcd for $C_{27}H_{18}N_3O$ 400.14499); purity 99.9% t_R = 6.59 min.

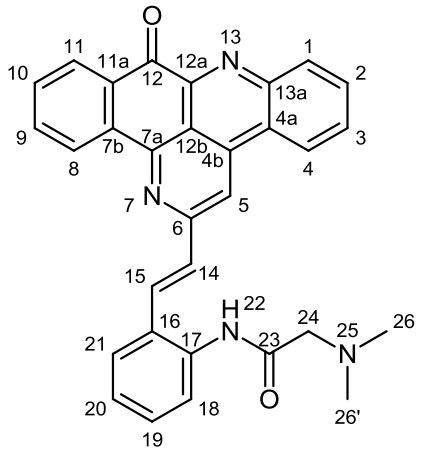
6-(2-Amino-E-styryl)-8-deaza-ascdidemin (**2.48**):



Prepared according to the general procedure using nitrostyryl **2.43** (46 mg, 0.11 mmol). Reaction workup was performed utilising standard conditions to give a red solid **2.48** (40 mg, 94 %).

m.p 266-267 ºC; IR ν_{max} 3445, 3330, 3225, 3057, 1671, 1638, 1627, 1589, 1569, 1488, 1269, 1053, 963, 763 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.78 (1H, dd, *J* = 8.0, 0.7 Hz, H-8), 8.47 (1H, dd, *J* = 9.1, 0.9 Hz, H-1), 8.46 (1H, dd, *J* = 9.0, 0.7 Hz, H-4), 8.38 (1H, dd, *J* = 7.8, 1.2 Hz, H-11), 8.16 (1H, d, *J* = 15.5 Hz, H-15), 7.99 (1H, s, H-5), 7.87 (1H, ddd, *J* = 8.4, 6.9, 1.2 Hz, H-2), 7.79 (1H, ddd, *J* = 8.2, 7.0, 1.0 Hz, H-3), 7.72 (1H, ddd, *J* = 7.7, 7.4, 1.3 Hz, H-9), 7.58 (1H, ddd, *J* = 7.7, 7.3, 1.1 Hz, H-10), 7.53 (1H, dd, *J* = 7.8, 1.2 Hz, H-21), 7.21 (1H, ddd, *J* = 7.9, 7.3, 1.4 Hz, H-19), 7.14 (1H, d, *J* = 15.4 Hz, H-14), 6.86 (1H, br t, *J* = 7.5 Hz, H-20), 6.82 (1H, dd, *J* = 7.9, 0.8 Hz, H-18), 4.23 (2H, s, H-22); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.3 (C-12), 154.8 (C-6), 149.8 (C-7a), 146.7 (C-12a), 146.0 (C-13a), 145.2 (C-17), 138.5 (C-4b), 136.0 (C-7b), 134.6 (C-9), 132.8 (C-1), 132.5 (C-11a), 131.4 (C-15), 131.3 (C-2), 131.0 (C-10), 130.0 (C-19), 129.9 (C-3), 128.5 (C-11), 127.8 (C-14), 127.6 (C-21), 125.6 (C-8), 123.6 (C-4a), 122.6 (C-4, C-16), 119.1 (C-20), 116.7 (C-18), 115.8 (C-12b), 113.0 (C-5); FABMS *m/z* 400 $[M+H]^+$; HRFABMS *m/z* 400.14533 (calcd for $C_{27}H_{18}N_3O$ 400.14499); purity 99.8% t_R = 6.90 min.

6-(2-N,N-Dimethylglycimidyl-E-styryl)-8-deaza-ascdidemin (**2.49**):

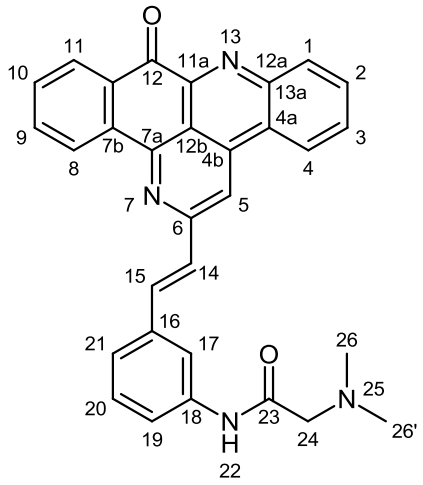


To a mixture of amine **2.48** (11.65 mg, 0.0292 mmol) and N,N-dimethylglycine hydrochloride (8.29 mg, 0.0594 mmol) in dichoromethane (1 mL) and dimethylformamide (0.3 mL) was added DCC (12.61 mg, 0.0611 mmol) in dichloromethane (1.0 mL) and the mixture was stirred under a nitrogen atmosphere for 66 hrs. The mixture was dried in vacuo and the resulting solid was repeatedly extracted with HCl (0.01 $molL^{-1}$), the resulting orange solution was filtered through cotton wool and loaded directly onto a reverse-phase C18 flash chromatography column. The column in turn was eluted with 70% methanol:water /0.1% HCl. The primary band (now yellow) was isolated and dried in vacuo to give a green solid **2.49** (15.1 mg, 99 %).

m.p. 202-203 ºC; IR ν_{max} 3368, 3042, 1696, 1670, 1587, 1554, 1457, 1401, 1022, 960, 759 cm^{-1}; ^{1}H NMR ($(CD_3)_2SO$, 400 MHz) δ 11.14 (1H, s, H-22), 10.32 (1H, s, H-25), 9.03 (1H, d, *J* = 7.8 Hz, H-4), 8.99 (1H, s, H-5), 8.83 (1H, d, *J* = 7.8 Hz, H-8), 8.36 (1H, d, *J* = 15.9 Hz, H-15), 8.29 (1H, d, *J* = 8.0 Hz, H-1), 8.19 (1H, d, *J* = 7.6 Hz, H-11), 7.93 (4H, m, *J* = Hz, H-2, 3, 9, 21), 7.70 (1H, t, *J* = 7.4 Hz, H-10), 7.58 (1H, d, *J* = 7.8 Hz, H-18), 7.44 (2H, m, H-14, 19), 7.36 (1H, t, *J* = 7.4 Hz, H-20), 4.47 (2H, d, *J* = 4.4 Hz, H-24), 2.96 (6H, d, *J* = 4.2 Hz, H-26, 26'); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz) δ 181.1 (C-12), 163.9 (C-23), 154.4 (C-6), 149.0 (C-7a), 146.3 (C-12a), 145.1 (C-13a), 138.1 (C-4b), 135.3 (C-7b), 134.83 (C-17), 134.76 (C-9), 132.1 (C-11a), 131.6 (C-2), 131.5 (C-1), 131.1 (C-10), 130.7 (C-16), 130.4 (C-15), 130.2 (C-3), 129.2 (C-14), 129.0 (C-19), 127.6 (C-11), 126.4 (C-20), 126.2 (C-18, C-21), 125.4 (C-8), 124.2 (C-4), 123.3 (C-4a), 115.5 (C-12b), 114.3 (C-5), 57.9 (C-24), 43.2 (C-26, C-26');

FABMS *m/z* 485 $[M+H]^+$; HRFABMS *m/z* 485.19767 (calcd for $C_{31}H_{25}N_4O_2$ 485.19775); purity (305 nm) 99.5 % t_R = 6.79 min.

6-(3-N,N-Dimethylglycimidyl-E-styryl)-8-deaza-ascdidemin (**2.50**):

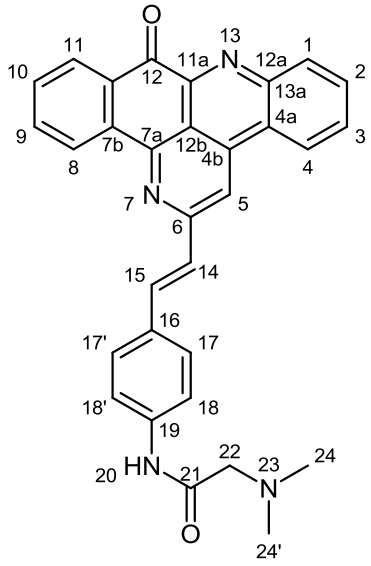


To a mixture of amine **2.46** (12.1 mg, 0.0300 mmol) and *N*,*N*-dimethylglycine hydrochloride (39.2 mg, 0.281 mmol) in dichoromethane (1 mL) and dimethylformamide (0.2 mL) was added DCC (64.5 mg, 0.313 mmol) in dichloromethane (1 mL) and the mixture was stirred under a nitrogen atmosphere for 20 hrs. The mixture was dried in vacuo and the resulting solid was repeatedly extracted with HCl (0.01 $molL^{-1}$), the resulting orange solution was filtered through cotton wool and loaded directly onto a reverse-phase C18 flash chromatography column. The column in turn was eluted with 70% methanol:water /0.1% HCl. The primary band (now yellow) was isolated and dried in vacuo to give a red solid **2.50** (8.75 mg, 55 %).

m.p. 210-211 ºC; IR (smear) ν_{max} 3370, 3069, 1689, 1674, 1589, 1565, 1401, 1272, 962, 770 cm^{-1}; ^1H NMR ($(CD_3)_2SO$, 400 MHz) δ ; 11.25 (1H, s, H-22), 10.27 (1H, s, H-25), 8.87 (1H, br d, J = 8.0 Hz, H-4), 8.81 (1H, br d, J = 7.8 Hz, H-8), 8.76 (1H, s, H-5), 8.31 (1H, br d, J = 7.9 Hz, H-1), 8.18 (1H, dd, J = 7.6, 0.7 Hz, H-11), 8.09 (1H, br s, H-17), 8.01 (1H, d, J = 15.9 Hz, H-15), 7.98 (1H, td, J = 7.6, 1.0 Hz, H-2), 7.92 (1H, m, H-3), 7.91 (1H, m, H-9), 7.71 (1H, td, J = 7.5, 0.8 Hz, H-10), 7.62 (1H, br d, J = 7.8 Hz, H-19), 7.51 (1H, br d, J = 7.8 Hz, H-21), 7.46 (1H, t, J = 7.6 Hz, H-20); 7.43 (1H, d, J = 15.9 Hz, H-14), 4.28 (2H, d, J = 4.1 Hz, H-24), 2.95 (6H, d, J = 4.0 Hz, H-

26, 26'); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz) δ 181.0 (C-12), 163.2 (C-23), 153.8 (C-6), 149.0 (C-7a), 146.3 (C-12a), 145.1 (C-13a), 138.6 (C-18), 138.1 (C-4b), 136.7 (C-16), 135.4 (C-7b), 134.7 (C-9), 134.3 (C-15), 132.2 (C-11a), 131.7 (C-2), 131.5 (C-1), 131.1 (C-10), 130.1 (C-3), 129.4 (C-20), 127.9 (C-14), 127.6 (C-11), 125.3 (C-8), 123.9 (C-4), 123.2 (C-21, C-4a), 120.0 (C-19), 118.0 (C-17), 115.6 (C-12b), 114.3 (C-5), 57.8 (C-24), 43.2 (C-26, C-26'); FABMS *m/z* 485 $[M+H]^+$; HRFABMS *m/z* 485.19851 (calcd for $C_{31}H_{25}N_4O_2$ 485.19775); purity (305 nm) 99.6 % t_R = 6.88 min.

6-(4-N,N-Dimethylglycimidyl-E-styryl)-8-deaza-ascdidemin (**2.51**):

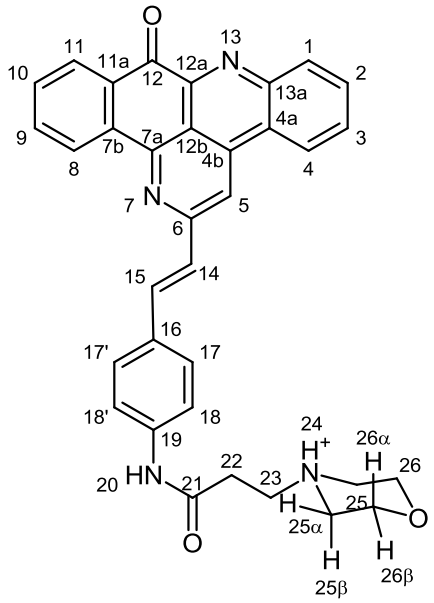


To a mixture of amine **2.47** (9.9 mg, 0.0248 mmol) and N,N-dimethylglycine hydrochloride (7.2 mg, 0.0517 mmol) in dichoromethane (1 mL) and dimethylformamide (0.3 mL) was added DCC (11.7mg, 0.566 mmol) in dichloromethane (1 mL) and the mixture was stirred under a nitrogen atmosphere for 66 hrs. The mixture was dried in vacuo and the resulting solid was repeatedly extracted with HCl (0.01 $molL^{-1}$), the resulting orange solution was filtered through cotton wool and loaded directly onto a reverse-phase C18 flash chromatography column. The column in turn was eluted with 70% methanol:water /0.1% HCl. The primary band (now yellow) was isolated and dried in vacuo to give a dark red solid **2.51** (10.1 mg, 78 %).

m.p. 213-214 ºC; IR (smear) ν_{max} 3335, 3044, 1697, 1677, 1649, 1613, 1586, 1562, 1536, 1510, 1415, 1313, 1255, 1056, 948 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 8.56

(1H, d, *J* = 7.0 Hz, H-4), 8.43 (1H, d, *J* = 7.5 Hz, H-8), 8.20 (1H, d, *J* = 8.2 Hz, H-1), 8.05 (1H, s, H-5), 7.99 (1H, d, *J* = 7.6 Hz, H-11), 7.88 (1H, t, *J* = 7.4 Hz, H-2), 7.81 (1H, t, *J* = 6.5 Hz, H-3), 7.70 (1H, t, *J* = 7.3 Hz, H-9), 7.64 (1H, d, *J* = 15.9 Hz, H-15), 7.56 (2H, m, H-18, 18'), 7.54 (1H, m, H-10), 7.41 (2H, d, *J* = 7.5 Hz, H-17, 17'), 6.99 (1H, d, *J* = 15.4 Hz, H-14), 4.26 (2H, s, H-22), 3.09 (6H, s, H-24, 24'); ^{13}C NMR (CD_3OD, 100 MHz) δ 180.1 (C-12), 164.0 (C-21), 158.8 (C-6), 152.4 (C-7a), 146.4 (C-12a), 141.1 (C-13a), 140.2 (C-19), 138.3 (C-4b), 138.2 (C-15), 136.9 (C-9), 136.3 (C-7b), 134.4 (C-2), 133.6 (C-16), 133.0 (C-10), 132.5 (C-11b), 132.3 (C-3), 129.9 (C-17, 17'), 129.3 (C-11), 128.2 (C-1), 127.0 (C-8, C-14), 125.8 (C-4a), 125.3 (C-4), 121.0 (C-18, C-18'), 115.3 (C-12b), 114.5 (C-5), 60.1 (C-22), 44.8 (C-24, C-24'); FABMS *m/z* 485 $[M+H]^+$; HRFABMS *m/z* 485.19720 (calcd for $C_{31}H_{25}N_4O_2$ 485.19775); purity (305 nm) 98.2 % t_R = 6.85 min.

6-(4-[[3-(4-Morpholinyl)-1-oxopropyl]amino]-E-styryl)-8-deaza-ascididemin hydrochloride salt. (**2.52**):

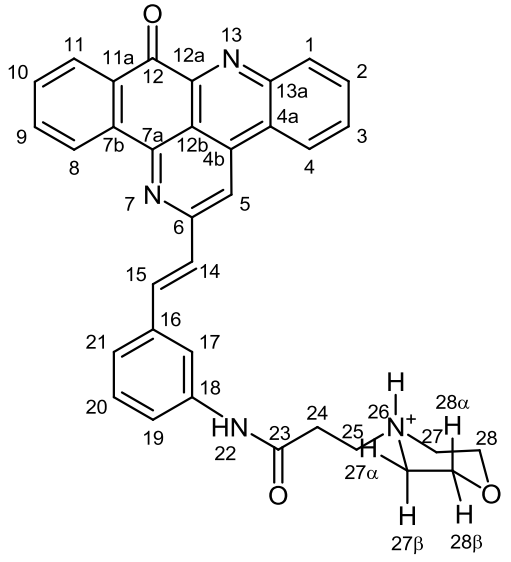


To a mixture of amine **2.47** (11.9 mg, 0.0298 mmol) and 4-(2-carboxyethyl)morpholine hydrochloride (**2.56**) (12.0 mg, 0.0611 mmol) in dichoromethane (1 mL) and dimethylformamide (0.3 mL) was added DCC (12.1 mg, 0.0586 mmol) in dichloromethane (1 mL) and the mixture was stirred under a nitrogen atmosphere for 65 hrs. The mixture was dried in vacuo and the resulting solid was repeatedly extracted with HCl (0.01 $molL^{-1}$), the resulting orange solution was

filtered through cotton wool and loaded directly onto a reverse-phase C18 flash chromatography column. The column in turn was eluted with 50% methanol:water /0.1% HCl. The primary band (now yellow) was isolated and dried in vacuo to give a red solid **2.52** (8.18 mg, 48 %).

m.p. 205-206 ºC; IR (smear) ν_{max} 3372, 3042, 1669, 1614, 1587, 1572, 1534, 1514, 1404, 1263, 1124, 965; ^{1}H NMR ($(CD_3)_2SO$, 400 MHz) δ 11.14 (1H, s, H-24), 10.62 (1H, s, H-20), 9.00 (1H, d, *J* = 7.9 Hz, H-8), 8.96 (1H, d, *J* = 7.7 Hz, H-4), 8.85 (1H, s, H-5), 8.41 (1H, d, *J* = 8.0 Hz, H-1), 8.30 (1H, d, *J* = 7.8 Hz, H-11), 8.17 (1H, d, *J* = 15.9 Hz, H-15), 8.06 (1H, t, *J* = 7.1 Hz, H-2), 8.00 (1H, t, *J* = 7.3 Hz, H-3), 7.99 (1H, t, *J* = 6.8 Hz, H-9), 7.81 (1H, t, *J* = 7.6 Hz, H-10), 7.77 (4H, m, H-17, 18), 7.56 (1H, d, *J* = 15.9 Hz, H-14), 3.98 (2H, obsc by h2o peak, H-26β), 3.82 (2H, t, *J* = 11.6 Hz, H-26α), 3.43 (4H, m, H-25α, 23), 3.13 (2H, m, H-25β), 3.02 (2H, t, *J* = 7.3 Hz, H-22); δ ^{13}C NMR ($(CD_3)_2SO$, 100 MHz) δ 181.2 (C-12), 167.8 (C-21), 154.5 (C-6), 149.3 (C-7a), 146.6 (C-12a), 145.3 (C-13a), 139.5 (C-19), 138.2 (C-4b), 135.6 (C-7b), 134.7 (C-9), 134.5 (C-15), 132.4 (C-11a), 131.7 (C-1, C-2), 131.2 (C-10, C-16), 130.2 (C-3), 128.0 (C-17), 127.7 (C-11), 126.2 (C-14), 125.4 (C-8), 123.8 (C-4), 123.3 (C-4a), 119.3 (C-18), 115.6 (C-12b), 113.9 (C-5), 63.1 (C-26), 51.7 (C-23), 51.1 (C-25), 30.2 (C-22); FABMS *m/z* 541 $[M+H]^+$; HRFABMS *m/z* 541.22546 (calcd for $C_{34}H_{29}N_4O_3$ 541.22397); purity (305 nm) 98.6 % t_R = 6.99 min.

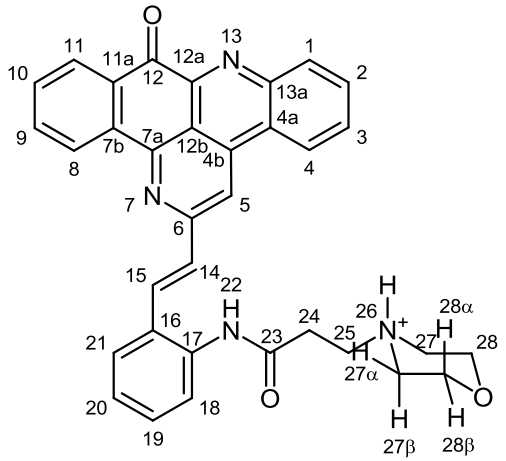
6-(3-[[3-(4-Morpholinyl)-1-oxopropyl]amino]-E-styryl)-8-deaza-asciidemin hydrochloride salt (**2.53**):



To a mixture of amine **2.46** (15.1 mg, 0.378 mmol) and 4-(2-carboxyethyl)morpholine hydrochloride (**2.56**) (14.7 mg, 0.0752 mmol) in dichoromethane (1 mL) and dimethylformamide (0.3 mL) was added DCC (16.6 mg, 0.0805 mmol) in dichloromethane (1 mL) and the mixture was stirred under a nitrogen atmosphere for 46 hrs. The mixture was dried in vacuo and the resulting solid was repeatedly extracted with HCl (0.01 $molL^{-1}$), the resulting orange solution was filtered through cotton wool and loaded directly onto a reverse-phase C18 flash chromatography column. The column in turn was eluted with 50% methanol:water /0.1% HCl. The primary band (now yellow) was isolated and dried in vacuo to give a red solid **2.53** (7.75 mg, 35 %).

m.p. 215-216 ºC; IR (smear) ν_{max} 3370, 3071, 1683, 1614, 1586, 1562, 1426, 1372, 1080, 981, 875 cm^{-1}; ^{1}H NMR ($(CD_3)_2SO$, 400 MHz) δ 11.24 (1H, s, H-26), 10.57 (1H, s, H-22), 8.96 (1H, d, *J* = 7.8 Hz, H-4), 8.93 (1H, d, *J* = 7.7 Hz, H-8), 8.89 (1H, s, H-5), 8.38 (1H, d, *J* = 7.9 Hz, H-1), 8.27 (1H, d, *J* = 7.3 Hz, H-11), 8.15 (1H, s, H-17), 8.11 (1H, d, *J* = 16.0 Hz, H-15), 8.04 (1H, t, *J* = 6.9 Hz, H-2), 7.98 (1H, t, *J* = 6.9 Hz, H-3), 7.96 (1H, t, *J* = 8.0 Hz, H-9), 7.77 (1H, t, *J* = 7.5 Hz, H-10), 7.55 (1H, m, H-19), 7.52 (1H, m, H-14), 7.49 (1H, m, H-21), 7.43 (1H, t, *J* = 7.7 Hz, H-20), 4.00 (2H, d, *J* = 11.4 Hz, H-28β), 3.84 (2H, t, *J* = 12.5 Hz, H-28α), 3.44 (4H, m, H-25, 27α), 3.15 (2H, m, H-25β), 3.04 (2H, t, *J* = 7.3 Hz, H-24); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz) δ 181.1 (C-12), 167.9 (C-23), 154.0 (C-6), 149.2 (C-7a), 146.5 (C-12a), 145.3 (C-13a), 139.4 (C-18), 138.2 (C-4b), 136.6 (C-16), 135.5 (C-7b), 134.8 (C-15), 134.6 (C-9), 132.3 (C-11a), 131.73 (C-2), 131.68 (C-1), 131.2 (C-10), 130.2 (C-3), 129.3 (C-20), 127.7 (C-14), 127.6 (C-11), 125.4 (C-8), 123.9 (C-4), 123.3 (C-4a), 122.7 (C-21), 119.8 (C-19), 117.8 (C-17), 115.7 (C-12b), 114.3 (C-5), 63.1 (C-28), 51.8 (C-25), 51.1 (C-27), 30.1 (C-24); FABMS *m/z* 541 $[M+H]^+$; HRFABMS *m/z* 541.22386 (calcd for $C_{34}H_{29}N_4O_3$ 541.22397); purity (305 nm) 95.6 % t_R = 7.04 min.

6-(2-[[3-(4-Morpholinyl)-1-oxopropyl]amino]-E-styryl)-8-deaza-ascididemin hydrochloride salt (**2.54**):

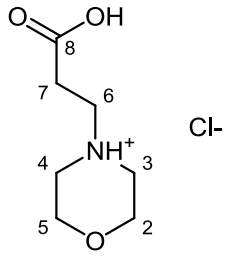


To a mixture of amine **2.48** (15.4 mg, 0.0386 mmol) and 4-(2-carboxyethyl)morpholine hydrochloride (**2.56**) (14.7 mg, 0.0751 mmol) in dichoromethane (1 mL) and dimethylformamide (0.3 mL) was added DCC (15.6 mg, 0.756 mmol) in dichloromethane (1 mL) and the mixture was stirred under a nitrogen atmosphere for 46 hrs. The mixture was dried in vacuo and the resulting solid was repeatedly extracted with HCl (0.01 $molL^{-1}$), the resulting orange solution was filtered through cotton wool and loaded directly onto a reverse-phase C18 flash chromatography column. The column in turn was eluted with 50% methanol:water /0.1% HCl. The primary band (now yellow) was isolated and dried in vacuo to give a green solid **2.54** (10.96 mg, 49 %).

m.p. 196-197 ºC; IR (smear) ν_{max} 3380, 3059, 1675, 1588, 1574, 1532, 1404, 1125, 981, 755 cm^{-1};^{1}H NMR ($(CD_3)_2SO$, 400 MHz) δ 11.59 (1H, s, H-26), 10.47 (1H, s, H-22), 9.01 (1H, d, *J* = 7.6 Hz, H-4), 8.95 (1H, s, H-5), 8.90 (1H, d, *J* = 7.6 Hz, H-8), 8.37 (1H, m, H-15), 8.34 (1H, m, H-1), 8.24 (1H, d, *J* = 7.6 Hz, H-11), 8.04 (1H, m, H-9), 8.01 (1H, m, H-2), 7.96 (1H, m, H-3), 7.91 (1H, d, *J* = 7.6 Hz, H-21), 7.74 (1H, t, *J* = 7.3 Hz, H-10), 7.56 (1H, d, *J* = 7.8 Hz, H-18), 7.50 (1H, d, *J* = 15.9 Hz, H-14), 7.41 (1H, t, *J* = 7.4 Hz, H-19), 7.33 (1H, t, *J* = 7.2 Hz, H-20), 3.94 (2H, d, *J* = 11.6 Hz, H-28β), 3.86 (2H, t, *J* = 11.8 Hz, H-28α), 3.50 (2H, m, H-25), 3.46 (2H, d, *J* = 13.7 Hz, H-27α), 3.23 (2H, t, *J* = 7.0 Hz, H-24), 3.13 (2H, q, *J* = 10.8 Hz, H-27β); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz) δ 181.1 (C-12), 168.4 (C-23), 154.5 (C-6), 149.1 (C-7a), 146.4 (C-12a), 145.1 (C-13a), 138.2 (C-4b), 135.9 (C-17), 135.5 (C-7b), 135.0 (C-9),

132.2 (C-11a), 131.7 (C-2), 131.5 (C-1), 131.1 (C-10), 130.7 (C-15), 130.5 (C-16), 130.2 (C-3), 128.9 (C-19), 128.7 (C-14), 127.6 (C-11), 126.5 (C-18), 126.1 (C-21), 125.9 (C-20), 125.4 (C-8), 124.1 (C-4), 123.3 (C-4a), 115.6 (C-12b), 114.3 (C-5), 63.1 (C-28), 51.9 (C-25), 51.1 (C-27), 29.7 (C-24); FABMS *m/z* 541 $[M+H]^+$; HRFABMS *m/z* 541.22378 (calcd for $C_{34}H_{29}N_4O_3$ 541.22397); purity (305 nm) 97.1 % t_R = 6.77 min.

4-(2-Carboxyethyl)morpholine hydrochloride (**2.56**):

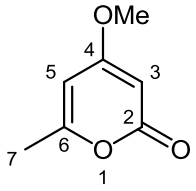


Preparation was achieved following a literature procedure.[92] To a solution of morpholine (280 μL, 3.18 mmol) in ether (16 mL) was added ethyl-3-bromo-propionate (200 μL, 1.57 mmol) in a dropwise fashion for 1 hr. The resultant suspension was cooled on ice over 1 hr, filtered and the filtrate was dried in vacuo. This solid was dissolved in HCl (15%) and stirred at 100 ºC for 6 hrs. The reaction mixture was dried in vacuo and recrystallised from methanol:acetonitrile (1:1) to give 4-(2-carboxyethyl)morpholine hydrochloride **2.56** as a white solid (138 mg, 0.71 mmol, 50 %).

^{1}H NMR (D_2O, 400 MHz) δ 3.88 (2H, d, *J* = 12.7 Hz, H-3,5 ax), 3.59 (2H, t, *J* = 12.2 Hz, H-3,5 eq), 3.31 (2H, d, *J* = 11.2 Hz, H-2,6 eq), 3.25 (2H, t, *J* = 7.0 Hz, H-7), 2.98 (2H, t, *J* = 10.4 Hz, H-2,6 ax), 2.66 (2H, t, *J* = 7.0 Hz, H-8); ^{13}C NMR (D_2O, 100 MHz) δ 174.6 (C-8), 64.5 (C-3, C-5), 53.4 (C-7), 52.7 (C-2, C-6), 29.1 (C-8); NMR was referenced to internal dioxane (δ_H 3.50 ppm, δ_C 67.4 ppm) consistent with published data;[92] FABMS *m/z* 160 $[M+H]^+$; HRFABMS *m/z* 160.09715 (calcd for $C_7H_{14}NO_3$ 160.09737).

4.3 Experimental details for Chapter 3

4-Methoxy-6-methyl-2-pyrone (**3.40**):



To a mixture of 4-hydroxy-6-methyl-2-pyrone (521 mg, 4.13 mmol) and potassium carbonate (692 mg, 8.59 mmol) was added trimethyl phosphate (1.01 mL, 8.59 mmol) and the solution was stirred at 140 ºC for 1 hr under nitrogen. The reaction mixture was cooled to room temperature and poured onto a mixture of water (20 mL) and ethyl acetate (40 mL). The layers were separated and the aqueous layer was further extracted with ethyl acetate (2 x 50 mL). The combined organic layers were dried with anhydrous magnesium sulphate followed by solvent removal in vacuo. The material was purified by silica gel chromatography (eluted with dichloromethane), and crystallised with ethyl acetate/hexane. This afforded **3.40** as a white crystalline material (407 mg, 70 %).

Mp 86-87 ºC (lit.[135] 86-87.5 ºC); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.71 (1H, d, *J* = 0.8 Hz,), 5.33 (1H, s), 3.72 (3H, s), 2.13 (3H, s); NMR ($CDCl_3$, 100 MHz) δ 171.2, 164.8, 161.9, 100.2, 87.1, 55.6, 19.6; FABMS *m/z* 141 $[M+H]^+$; HRFABMS *m/z* 141.05541 (calcd for $C_7H_9O_3$ 141.05517).

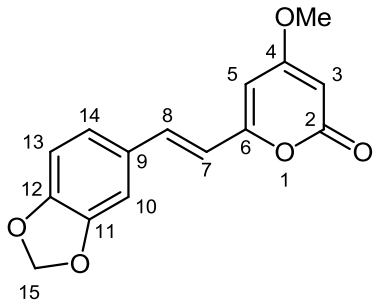
General reaction procedure used for the preparation of styryl pyrones **3.36**, **3.39**, **3.42-48**, **3.50-56**:

4-Methoxy-6-methyl-2-pyrone (200 mg, 1.4 mmol) and aldehyde (1.7 mmol) were added in methanol (5 mL) to a gently refluxing suspension of magnesium methoxide (freshly prepared from 104 mg magnesium by gently heating in anhydrous methanol) in methanol (5 mL) under nitrogen. The reaction mixture was refluxed for 6 hrs and then allowed to cool. The cooled mixture was dried in vacuo and the solid treated with

acetic acid (3.3 M, ~20 mL) and extracted with dichloromethane (4 x 50 mL), the combined organic layers were washed with water (2 x 50 mL) and dried in vacuo. Unless otherwise stated purification of the resultant pyrone was achieved first by precipitation of oil (where crude oils resulted) with ether followed by recrystallisation in methanol.

A modified methodology was developed, whereby the reaction vessel was surrounded in aluminium foil and exposure to light was minimised during workup and recrystallisation, resulted in greatly improved yields. Samples for which this methodology was applied are noted with the improved yield stated.

6-[2-(1,3-Benzodioxol-5-yl)ethenyl]-4-methoxy-2H-pyran-2-one (**3.36**):

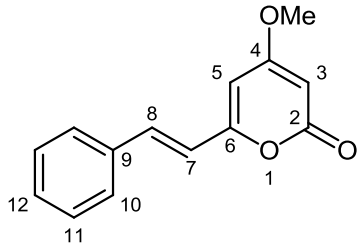


Pyrone (**3.36**) was prepared and purified under standard conditions from pyrone **3.40** (202 mg, 1.4 mmol) and piperanal (258 mg, 1.7 mmol). Recrystallisation from methanol afforded **3.36** as a yellow crystalline solid (78 mg, 20 %). Use of the Light exclusion methodology with pyrone **3.40** (201 mg, 1.4 mmol) and piperanal (260 mg, 1.7 mmol) gave **3.36** in higher yield (155 mg, 40%).

Mp 231-232 ºC (lit.[136] 232-233ºC); TLC R_f (3% MeOH:CH_2Cl_2) 0.43; IR (smear) ν_{max} 3076, 1716, 1639, 1618, 1550, 1498, 1449, 1409, 1360, 1240, 1153, 1026, 953, 923, 831, 795 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.40 (1H, d, *J* = 15.9, H-8), 7.00 (1H, d, *J* = 1.6 Hz, H-10), 6.96 (1H, dd, *J* = 8.2, 1.7 Hz, H-14), 6.80 (1H, d, *J* = 8.0 Hz, H-13), 6.39 (1H, d, *J* = 15.9 Hz, H-7), 5.99 (2H, s, H-15), 5.89 (1H, d, *J* = 2.2 Hz, H-5), 5.47 (1H, d, *J* = 2.2 Hz, H-3), 3.81 (3H, s, OMe); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.1 (C-4), 164.0 (C-2), 158.8 (C-6), 148.9 (C-12), 148.3 (C-11), 135.5 (C-8), 129.7 (C-9), 123.5 (C-14), 116.8 (C-7), 108.6 (C-10), 105.9 (C-13), 101.4 (C-15), 100.7 (C-5), 88.5 (C-3), 55.9 (OMe); EIMS *m/z* 272 $[M]^+$; HREIMS *m/z* 272.06861 (calcd for

$C_{15}H_{12}O_5$ 272.06847); Anal. Calcd for $C_{14}H_{12}O_3$: C, 66.17; H, 4.44. Found: C, 66.12; H, 4.53.

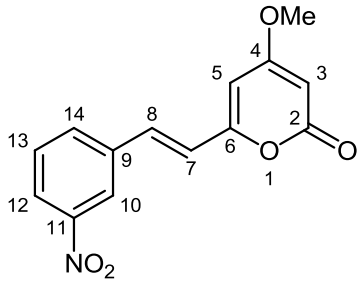
4-Methoxy-6-styryl-2H-pyran-2-one (**3.39**):



Pyrone (**3.39**) was prepared and purified under standard conditions from pyrone **3.40** (200 mg, 1.4 mmol) and benzaldehyde (175 μL, 1.7 mmol). Recrystallisation in methanol afforded **3.39** as a white microcrystalline solid (60 mg, 19 %). Light exclusion methodology with the pyrone (200 mg, 1.4 mmol) and benzaldehyde (180 μL, 1.8 mmol) gave a higher yield of identical product (153.1 mg, 47%).

Mp 137-138 ºC (lit.[112] 137-138 ºC); TLC R_f (3% MeOH:CH_2Cl_2) 0.78; IR (smear) ν_{max} 3076, 1719, 1635, 1607, 1555, 1446, 1406, 1256, 1152, 1001, 954, 831, 748, 685 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.49 (1H, obsc, H-8), 7.49 (2H, m, H-10), 7.35 (3H, m, H-11, H12), 6.56 (1H, d, *J* = 16.0 Hz, H-7), 5.93 (1H, d, *J* = 2.2 Hz, H-5), 5.50 (1H, d, *J* = 2.2 Hz, H-3), 3.83 (3H, s, H-OMe); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.1 (C-4), 164.0 (C-2), 158.6 (C-6), 135.8 (C-8), 135.2 (C-9), 129.4 (C-12), 128.9 (C-11), 127.4 (C-10), 118.6 (C-7), 101.3 (C-5), 88.9 (C-3), 55.9 (OMe); EIMS *m/z* 228 $[M]^+$; HREIMS *m/z* 228.07884 (calcd for $C_{14}H_{12}O_3$ 228.07864); Anal. Calcd for $C_{14}H_{12}O_3$: C, 73.67; H, 5.30. Found: C, 73.63; H, 5.44.

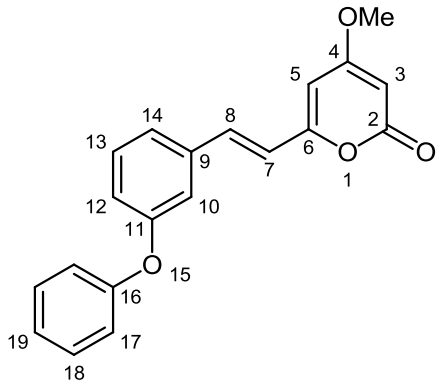
4-Methoxy-6-(3-nitrostyryl)-2H-pyran-2-one (**3.42**):



Pyrone (**3.42**) was prepared and purified under standard conditions from pyrone **3.40** (202 mg, 1.4 mmol) and 3-nitrobenzaldehyde (259 mg, 1.7 mmol). Recrystallisation in methanol afforded **3.42** as a yellow amorphous solid (115 mg, 29 %).

Mp 235-236 ºC (lit.[137] 238-240ºC); TLC R_f (3% MeOH:CH_2Cl_2) 0.76; IR (smear) ν_{max} 3078, 1726, 1611, 1558, 1526, 1454, 1415, 1347, 1257, 1155, 960, 839, 810, 734 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.37 (1H, t, *J* = 1.8 Hz, H-10), 8.17 (1H, ddd, *J* = 8.1, 2.2, 1.0 Hz, H-12), 7.75 (1H, bd, *J* = 7.7 Hz, H-14), 7.55 (1H, t, *J* = 8.0 Hz, H-13), 7.52 (1H, d, *J* = 16.0 Hz, H-8), 6.70 (1H, d, *J* = 16.0 Hz, H-7), 6.02 (1H, d, *J* = 2.2 Hz, H-5), 5.53 (1H, d, *J* = 2.2 Hz, H-3), 3.84 (3H, s, OMe); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.7 (C-4), 163.5 (C-2), 157.4 (C-6), 148.8 (C-11), 137.0 (C-9), 133.6 (C-14), 132.9 (C-8), 130.0 (C-13), 123.6 (C-12), 121.5 (C-7), 121.1 (C-10), 102.9 (C-5), 89.7 (C-3), 56.1 (OMe); EIMS *m/z* 273 $[M]^+$; HREIMS *m/z* 273.06367 (calcd for $C_{14}H_{11}NO_5$ 273.06372); Anal. Calcd for $C_{14}H_{11}NO_5.1/4.H_2O$: C, 60.54; H, 4.17; N, 5.04. Found: C, 60.52; H, 4.10; N, 4.92.

4-Methoxy-6-(3-phenoxy-styryl)-2H-pyran-2-one (**3.43**):

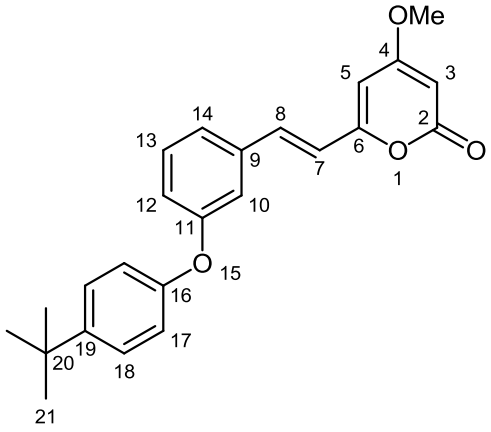


Pyrone (**3.43**) was prepared and worked up by standard methodology from pyrone **3.40** (200 mg, 1.4 mmol) and 3-phenoxy-benzaldehyde (295 μL, 1.7 mmol). The resulting oil was purified via repeated silica flash chromatography (dichloromethane and 1.0 % methanol in dichloromethane) to give a yellow oil. Solidification at 4 ºC overnight afforded **3.43** as a white solid (104 mg, 23 %).

Mp 85-86 ºC; TLC R_f (2% MeOH:CH_2Cl_2) 0.58; IR (smear) ν_{max} 3078, 1721, 1641, 1607, 1554, 1484, 1450, 1408, 1252, 1233, 1208, 1151, 955, 774, 691 cm^{-1}; ^1H NMR

($CDCl_3$, 400 MHz) δ 7.42 (1H, d, *J* = 15.9 Hz, H-8), 7.35 (2H, t, *J* = 7.5 Hz, H-18), 7.32 (1H, t, *J* = 8.0 Hz, H-13), 7.21 (1H, d, *J* = 7.9 Hz, H-14), 7.13 (1H, m, H-19), 7.11 (1H, m, H-10), 7.01 (2H, d, *J* = 7.8 Hz, H-17), 6.97 (1H, d, *J* = 8.2 Hz, H-12), 6.51 (1H, d, *J* = 16.0 Hz, H-7), 5.92 (1H, d, *J* = 2.0 Hz, H-5), 5.48 (1H, d, *J* = 2.0 Hz, H-3), 3.81 (3H, s, OMe); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.0 (C-4), 163.8 (C-2), 158.3 (C-6), 157.9 (C-11), 156.7 (C-16), 137.0 (C-9), 135.1 (C-8), 130.2 (C-13), 129.9 (C-18), 123.6 (C-19), 122.5 (C-14), 119.7 (C-12), 119.3 (C-7), 119.1 (C-17), 116.9 (C-10), 101.6 (C-5), 89.0 (C-3), 55.9 (OMe); EIMS *m/z* 320 $[M]^+$; HREIMS *m/z* 320.10508 (calcd for $C_{20}H_{16}O_4$ 320.10486); Anal. Calcd for $C_{20}H_{16}O_4.1/5H_2O$: C, 74.15; H, 5.10. Found: C, 74.07; H, 5.10.

4-Methoxy-6-(3-(4-tertbutyl-phenoxy)-styryl)-2H-pyran-2-one (**3.44**):

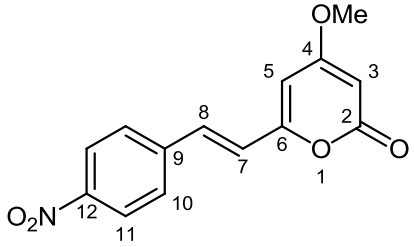


Pyrone (**3.44**) was prepared and purified under standard conditions from pyrone **3.40** (200 mg, 1.4 mmol) and 3-(4-*tert*-butyl-phenoxy)-benzaldehyde (436 mg, 1.7 mmol). Repeated recrystallisation from methanol afforded **3.44** as a white microcrystalline solid (20 mg, 3.7 %). Repeating the reaction but now taking care to exclude light, using pyrone (202 mg, 1.4 mmol) and 3-(4-*tert*-butyl-phenoxy)-benzaldehyde (434 mg, 1.7 mmol) gave the desired product (121 mg, 22%).

Mp 138-139 ºC; TLC R_f (2% MeOH:CH_2Cl_2) 0.64; IR (smear) ν_{max} 2959, 1717, 1638, 1565, 1505, 1442, 1242, 1215, 1174, 1146, 956, 830, 807 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.42 (1H, d, *J* = 16.0 Hz, H-8), 7.36 (2H, d, *J* = 8.8 Hz, H-18), 7.31 (1H, t, *J* = 7.9 Hz, H-13), 7.20 (1H, d, *J* = 7.9 Hz, H-14), 7.10 (1H, br s, H-10), 6.97 (1H, m, H-12), 6.95 (2H, d, *J* = 8.8 Hz, H-17), 6.52 (1H, d, *J* = 15.9 Hz, H-7), 5.92 (1H, d, *J* =

2.1 Hz, H-5), 5.48 (1H, d, *J* = 2.2 Hz, H-3), 3.81 (3H, s, OMe), 1.33 (9H, s, H-21); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.0 (C-4), 163.9 (C-2), 158.3 (C-6), 158.2 (C-11), 154.1 (C-16), 146.6 (C-19), 136.9 (C-9), 135.2 (C-8), 130.1 (C-13), 126.7 (C-18), 122.0 (C-14), 119.4 (C-12), 119.2 (C-7), 118.7 (C-17), 116.8 (C-10), 101.6 (C-5), 88.9 (C-3), 55.9 (OMe), 34.3 (C-20), 31.5 (C-21); EIMS *m/z* 376 $[M]^+$; HREIMS *m/z* 376.16741 (calcd for $C_{24}H_{24}O_4$ 376.16746); Anal. Calcd for $C_{24}H_{24}O_4$: C, 76.57; H, 6.43. Found: C, 76.69; H, 6.58.

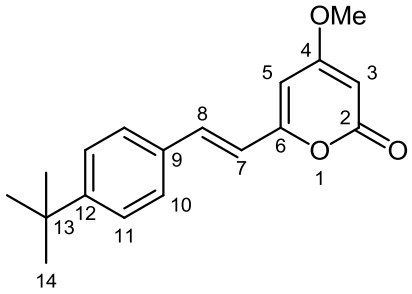
4-Methoxy-6-(4-nitrostyryl)-2H-pyran-2-one (**3.45**):



Pyrone (**3.45**) was prepared by standard methodology from pyrone **3.40** (199 mg, 1.4 mmol) and 4-nitrobenzaldehyde (258 mg, 1.7 mmol). Purification was achieved via repeated silica flash chromatography (dichloromethane and 1.0 % methanol in dichloromethane) followed by recrystallisation in methanol to afford **3.45** as a orange solid (21 mg, 5.3%).

Mp 213-214 ºC (lit[119] mp 211.5-214 ºC); TLC R_f (3% MeOH:CH_2Cl_2) 0.77; IR (smear) ν_{max} 3081, 1694, 1610, 1591, 1553, 1513, 1448, 1337, 1254, 1154, 955, 810 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.23 (2H, d, *J* = 8.8 Hz, H-11), 7.62 (2H, d, *J* = 8.8 Hz, H-10), 7.51 (1H, d, *J* = 16.0 Hz, H-8), 6.70 (1H, d, *J* = 16.0 Hz, H-7), 6.03 (1H, d, *J* = 2.1 Hz, H-5), 5.54 (1H, d, *J* = 2.1 Hz, H-3), 3.84 (3H, s, OMe); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.6 (C-4), 163.4 (C-2), 157.3 (C-6), 147.8 (C-12), 141.4 (C-9), 132.9 (C-8), 127.9 (C-10), 124.2 (C-11), 122.6 (C-7), 103.3 (C-5), 89.9 (C-3), 55.1 (OMe); EIMS *m/z* 273 $[M]^+$; HREIMS *m/z* 273.06323 (calcd for $C_{14}H_{11}NO_5$ 273.06372); Anal. Calcd for $C_{14}H_{11}NO_5.1/2H_2O$: C, 59.58; H, 4.29; N, 4.96. Found: C, 59.29; H, 4.46; N, 5.08.

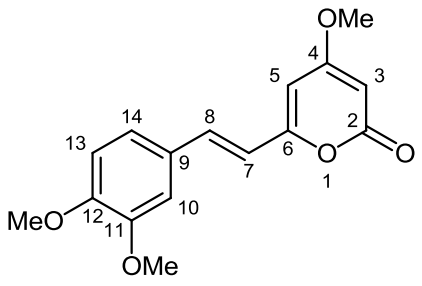
4-Methoxy-6-(4-tertbutyl-styryl)-2H-pyran-2-one (**3.46**):



Pyrone (**3.46**) was prepared and purified under standard conditions from pyrone **3.40** (201 mg, 1.4 mmol) and 4-*tert*-butyl-benzaldehyde (290 μL, 1.7 mmol). Repeated recrystallisation from methanol afforded **3.46** as a white microcrystalline solid (22 mg, 5.4 %). Application of the modified light exclusion methodology with pyrone (201 mg, 1.4 mmol) and 4-*tert*butyl-benzaldehyde (290 μL, 1.7 mmol) gave product (104 mg, 25%).

Mp 146-147 ºC; TLC R_f (3% MeOH:CH_2Cl_2) 0.77; IR (smear) ν_{max} 2960, 2870, 1713, 1638, 1552, 1450, 1404, 1247, 1151, 965, 824, 800 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.48 (1H, d, *J* = 16.0 Hz, H-8), 7.43 (2H, d, *J* = 8.5 Hz, H-10), 7.39 (2H, d, *J* = 8.4 Hz, H-11), 6.54 (1H, d, *J* = 15.9 Hz, H-7), 5.92 (1H, d, *J* = 2.4 Hz, H-5), 5.47(1H, d, *J* = 2.0 Hz, H-3), 3.81 (3H, s, OMe), 1.32 (9H, s, H-14); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.1 (C-4), 164.1 (C-2), 158.9 (C-6), 152.9 (C-12), 135.7 (C-7), 132.4 (C-9), 127.3 (C-10), 125.9 (C-11), 117.8 (C-7), 100.9 (C-5), 88.6 (C-3), 55.9 (OMe), 34.8 (C-13), 31.2 (C-14); EIMS *m/z* 284 $[M]^+$; HREIMS *m/z* 284.14116 (calcd for $C_{18}H_{20}O_3$ 284.14124); Anal. Calcd for $C_{18}H_{20}O_3$: C, 76.03; H, 7.09. Found: C, 75.87; H, 7.35.

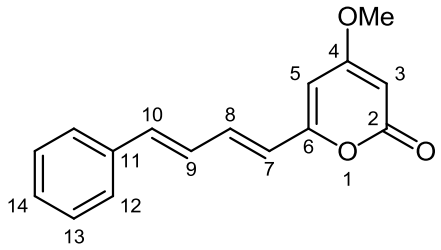
6-[2-(3,4-Dimethoxyphenyl)ethenyl]-4-methoxy-2H-pyran-2-one (**3.47**):



Pyrone (**3.47**) was prepared and purified under standard conditions, modified for light exclusion, from pyrone **3.40** (201 mg, 1.4 mmol) and 3,4-dimethoxybenzaldehyde (285 mg, 1.7 mmol). Repeated recrystallisation from methanol afforded **3.47** as a yellow crystalline solid (115 mg, 28 %).

Mp 162-163 ºC (lit.[118] 160-162 ºC); TLC R_f (3% MeOH:CH_2Cl_2) 0.42; IR (smear) 2945, 2837, 1700, 1638, 1595, 1550, 1516, 1449, 1408, 1252, 1230, 1153, 1032, 1018, 1003, 955, 838, 813, 802, 755 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.42 (1H, d, *J* = 15.9 Hz, H-8), 7.05 (1H, dd, *J* = 8.3, 1.9 Hz, H-14), 7.00 (1H, d, *J* = 1.9 Hz, H-10), 6.84 (1H, d, *J* = 8.3 Hz, H-13), 6.43 (1H, d, *J* = 15.8 Hz, H-7), 5.88 (1H, d, *J* = 2.2 Hz, H-5), 5.44 (1H, d, *J* = 2.2 Hz, H-3), 3.90 (3H, s, OMe-11), 3.88 (3H, s, OMe-12), 3.79 (3H, s, OMe-4);^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.2 (C-4), 164.1 (C-2), 158.9 (C-6), 150.4 (C-12), 149.2 (C-11), 135.6 (C-8), 128.2 (C-9), 121.6 (C-14), 116.5 (C-7), 111.2 (C-13), 109.3 (C-10), 100.5 (C-5), 88.4 (C-3), 55.9 (OMe), 55.8 (OMe); (+)-ESIMS *m/z* 289 $[M+H]^+$, (+)-HRESIMS *m/z* 289.1070 (calcd for $C_{16}H_{17}O_5$ 289.1071).

4-Methoxy-6-(4-phenyl-1,3-butadienyl)-2H-pyran-2-one (**3.48**):

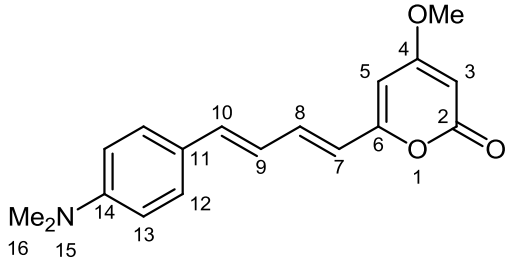


Pyrone (**3.48**) was prepared by standard methodology from pyrone **3.40** (201 mg, 1.4 mmol) and cinnamaldehyde (450 μL, 3.6 mmol). Purification was achieved via silica flash chromatography (dichloromethane and 1.0 % methanol in dichloromethane) followed by recrystallisation from methanol to afford **3.48** as a yellow solid (23 mg, 6.4 %).

Mp 189-190 ºC (lit.[110] 188.5-190 ºC); TLC R_f (3% MeOH:CH_2Cl_2) 0.81; IR (smear) ν_{max} 3077, 1722, 1630, 1547, 1451, 1410, 1260, 990, 956, 831, 743, 682 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.44 (2H, d, *J* = 7.2 Hz, H-12), 7.34 (2H, t, *J* = 7.1 Hz, H-

13), 7.30 (2H, m, H-14, H-8), 6.85 (1H, m, H-9), 6.83 (1H, m, H-10), 6.14 (1H, d, *J* = 15.2 Hz, H-7), 5.86 (1H, d, *J* = 2.1 Hz, H-5), 5.46 (1H, d, *J* = 2.2 Hz, H-3), 3.80 (3H, s, OMe), ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.0 (C-4), 164.0 (C-2), 158.7 (C-6), 138.3 (C-10), 136.4 (C-11), 136.3 (C-8), 128.8 (C-13), 128.6 (C-14), 127.1 (C-9), 127.0 (C-12), 122.0 (C-7), 100.9 (C-5), 88.7 (C-3), 55.9 (OMe); EIMS *m/z* 254 $[M]^+$; HREIMS *m/z* 254.09357 (calcd for $C_{16}H_{14}O_3$ 254.09429); Anal. Calcd for $C_{14}H_{12}O_3$: C, 75.57; H, 5.55. Found: C, 75.28; H, 5.68.

4-Methoxy-6-(4-(4-dimethylaminophenyl)-1,3-butadienyl)-2H-pyran-2-one (**3.49**):

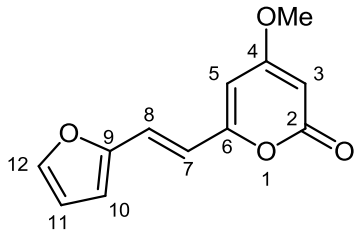


Pyrone (**3.49**) was prepared via modified methodology whereby 4-methoxy-6-methyl-2-pyrone **3.40** (200 mg, 1.4 mmol) and 4-dimethylamino-cinnamaldehyde (1.7 mmol) were added in methanol:dicholoromethane (3 mL:2 mL) to a gently refluxing suspension of magnesium methoxide (made from 104 mg magnesium) in methanol (5 mL) under nitrogen. The reaction mixture was refluxed for 22 hrs and then allowed to cool. The cooled mixture was dried in vacuo, the solid treated with acetic acid (3.3 M) and extracted with dichloromethane (4 x 50 mL). The combined organic layers were washed with water (2 x 50 mL) and dried in vacuo. Recrystallisation from methanol afforded **3.49** as a red solid (21 mg, 5.0 %).

Mp 239-240 ºC; TLC R_f (3% MeOH:CH_2Cl_2) 0.86; IR (smear) ν_{max} 3080, 2887, 1720, 1630, 1595, 1541, 1523, 1409, 1258, 1136, 986, 956 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33 (2H, d, *J* = 8.8 Hz, H-12), 7.28 (1H, dd, *J* = 15.2, 10.4 Hz, H-8), 6.75 (1H, m, H-10), 6.70 (1H, m, H-9), 6.65 (2H, d, *J* = 8.8 Hz, H-13), 6.02 (1H, d, *J* = 15.1 Hz, H-7), 5.79 (1H, d, *J* = 2.0 Hz, H-5), 5.41 (1H, d, *J* = 2.0 Hz, H-3), 3.78 (3H, s, OMe), 2.98 (6H, s, H-16); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.2 (C-4), 164.3 (C-2), 159.4 (C-6), 150.7 (C-14), 139.1 (C-10), 137.4 (C-8), 128.4 (C-12), 124.6 (C-11), 122.7 (C-9), 119.2 (C-7), 112.1 (C-13), 99.7 (C-5), 87.9 (C-3), 55.8 (OMe), 40.2 (C-

16); EIMS *m/z* 297 $[M]^+$; HREIMS *m/z* 297.13649 (calcd for $C_{18}H_{19}NO_3$ 297.13649); Anal. Calcd for $C_{18}H_{19}NO_3.1/10H_2O$: C, 72.27; H, 6.47; N, 4.68. Found: C, 72.16; H, 6.41; N, 4.65.

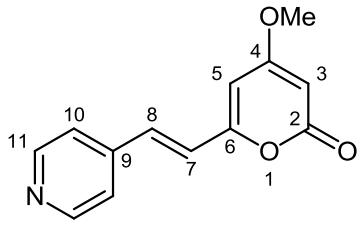
4-Methoxy-6-[2-(2-furyl)ethenyl]-2H-pyran-2-one (**3.50**):



Pyrone (**3.50**) was prepared and purified under standard conditions from pyrone **3.40** (200 mg, 1.4 mmol) and furfuraldehyde (145 μL, 1.8 mmol). Recrystallisation from methanol afforded **3.50** as a yellow solid (66 mg, 21 %).

Mp 174-175 ºC; TLC R_f (3% MeOH:CH_2Cl_2) 0.46; IR (smear) ν_{max} 3124, 3091, 2950, 2917, 2849, 1697, 1634, 1543, 1477, 1449, 1406, 1385, 1340, 1247, 1145, 998, 943, 809, 755 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.43 (1H, d, *J* = 1.5 Hz, H-12), 7.23 (1H, d, *J* = 15.6 Hz, H-8), 6.49 (1H, m, H-10), 6.47 (1H, m, H-7), 6.44 (1H, m, H-11), 5.89 (1H, d, *J* = 2.1 Hz, H-5), 5.45 (1H, d, *J* = 2.3 Hz, H-3), 3.81 (3H, s, OMe); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.0 (C-4), 163.9 (C-2), 158.4 (C-6), 151.6 (C-9), 144.0 (C-12), 122.6 (C-8), 116.5 (C-7), 113.3 (C-10), 112.3 (C-11), 101.2 (C-5), 88.7 (C-3), 55.9 (OMe); EIMS *m/z* 218 $[M]^+$; HREIMS *m/z* 218.05750 (calcd for $C_{12}H_{10}O_4$ 218.05791); Anal. Calcd for $C_{12}H_{10}O_4$: C, 66.05; H, 4.62. Found: C, 66.34; H, 4.85.

4-Methoxy-6-[2-(4-pyridinyl)ethenyl]-2H-pyran-2-one (**3.51**):

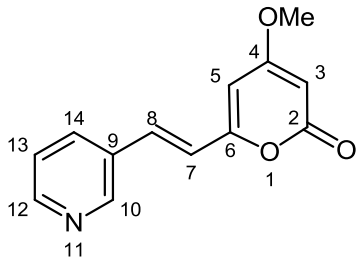


Pyrone (**3.51**) was prepared and purified under standard conditions from pyrone **3.40** (201 mg, 1.4 mmol) and 4-pyridine carboxaldehyde (170 μL, 1.8 mmol).

Recrystallisation from methanol afforded **3.51** as an acetate salt. This was dissolved in dichloromethane (10 mL), washed with 0.1 M aqueous sodium hydroxide (2 x 10 mL) and water (2 x 10 mL), and the dichloromethane was removed in vacuo to afford a white solid (12 mg, 3.5 %).

Mp 154-155 ºC (lit.[110] 146-150 ºC); TLC R_f (5% MeOH:CH_2Cl_2) 0.59; IR (smear) ν_{max} 3076, 1728, 1643, 1610, 1594, 1559, 1548, 1442, 1407, 1258, 1157, 1148, 1025, 999, 947, 837, 809, 689 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.60 (2H, dd, *J* = 4.6, 1.4 Hz, H-11), 7.38 (1H, d, *J* = 15.9 Hz, H-8), 7.31 (2H, dd, *J* = 4.6, 1.3 Hz, H-10), 6.72 (1H, d, *J* = 16.0 Hz, H-7), 6.01 (1H, d, *J* = 2.0 Hz, H-5), 5.52 (1H, d, *J* = 2.0 Hz, H-3), 3.82 (3H, s, OMe); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.6 (C-4), 163.4 (C-2), 157.2 (C-6), 150.5 (C-11), 142.3 (C-9), 132.8 (C-8), 122.8 (C-7), 121.3 (C-10), 103.2 (C-5), 89.8 (C-3), 56.1 (OMe); EIMS *m/z* 229 $[M]^+$; HREIMS *m/z* 229.07348 (calcd for $C_{13}H_{11}NO_3$ 229.07389); purity 97.8% t_R = 5.85 min; Anal. Calcd for $C_{13}H_{11}NO_3.1/3H_2O$: C, 66.38; H, 5.00; N, 5.95. Found: C, 66.55; H, 5.01; N, 5.92.

4-Methoxy-6-[2-(3-pyridinyl)ethenyl]-2H-pyran-2-one (**3.52**):

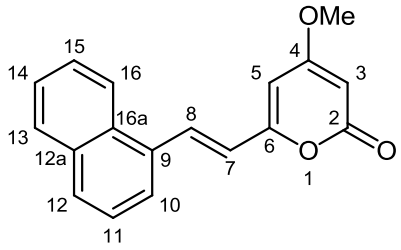


Pyrone (**3.52**) was prepared and purified under standard conditions from pyrone **3.40** (201 mg, 1.4 mmol) and 4-pyridine carboxaldehyde (165 μL, 1.7 mmol). Recrystallisation from methanol afforded **3.52** as a tan amorphous solid (29 mg, 8.8 %).

Mp 182-183 ºC (lit.[110] 180.5-182 ºC); TLC R_f (5% MeOH:CH_2Cl_2) 0.31; IR (smear) ν_{max} 3075, 1723, 1638, 1556, 1456, 1413, 1258, 1150, 950, 831, 799, 702 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) 8.72 (1H, d, *J* = 1.6 Hz, H-10), 8.54 (1H, dd, *J* = 4.7, 1.2 Hz, H-12), 7.77 (1H, dt, *J* = 7.9, 1.9 Hz, H-14), 7.45 (1H, d, *J* = 16.0 Hz, H-8), 7.29 (1H, dd, *J* = 7.9, 4.8 Hz, H-13), 6.63 (1H, d, *J* = 16.1 Hz, H-7), 5.98 (1H, d, *J* = 2.2

Hz, H-5), 5.51 (1H, d, *J* = 2.1, H-3), 3.82 (3H, s, OMe); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.8 (C-4), 163.6 (C-2), 157.7 (C-6), 150.1 (C-12), 149.0 (C-10), 133.7 (C-14), 132.0 (C-8), 131.0 (C-9), 123.7 (C-13), 120.5 (C-7), 102.2 (C-5), 89.4 (C-3), 56.0 (OMe); EIMS *m/z* 229 $[M]^+$; HREIMS *m/z* 229.07372 (calcd for $C_{13}H_{11}NO_3$ 229.07389); purity 99.7% t_R = 5.92 min; Anal. Calcd for $C_{13}H_{11}NO_3.1/5H_2O$: C, 67.06; H, 4.94; N, 6.02. Found: C, 67.04; H, 4.99; N, 6.03.

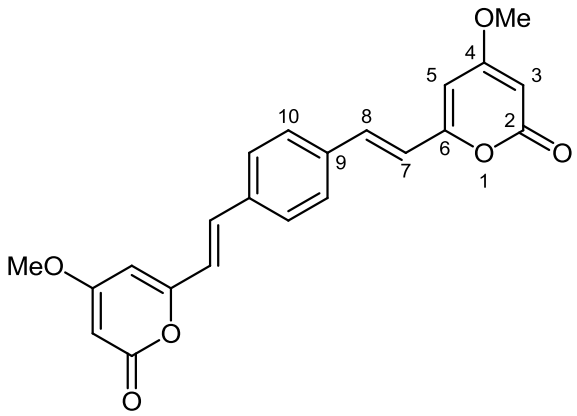
4-Methoxy-6-[2-(1-naphthyl)ethenyl]-2H-pyran-2-one (**3.53**):



Pyrone (**3.53**) was prepared and purified under standard conditions from pyrone **3.40** (200 mg, 1.4 mmol) and 1-napthaldehyde (233 μL, 1.7 mmol). Repeated recrystallisation from methanol afforded **3.53** as a yellow solid (35 mg, 8.8 %). Application of light exclusion methodology with the pyrone (200 mg, 1.4 mmol) and 1-napthaldehyde (233 μL, 1.7 mmol) gave **3.53** in higher yield (160 mg, 40%).

Mp 120-121 ºC; TLC R_f (3% MeOH:CH_2Cl_2) 0.83; IR (smear) ν_{max} 3079, 1724, 1636, 1556, 1448, 1411, 1263, 1155, 955, 763 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.29 (1H, d, *J* = 15.7 Hz, H-8), 8.23 (1H, d, *J* = 8.2 Hz, H-16), 7.85 (2H, m, H-12, 13), 7.72 (1H, d, *J* = 7.2 Hz, H-10), 7.58 (1H, ddd, *J* = 8.4, 6.8, 1.5 Hz, H-15), 7.52 (1H, ddd, *J* = 7.8, 7.1, 1.5 Hz, H-14), 7.47 (1H, t, *J* = 7.7 Hz, H-11), 6.66 (1H, d, *J* = 15.7 Hz, H-7), 5.98 (1H, d, *J* = 2.2 Hz, H-5), 5.53 (1H, d, *J* = 2.1 Hz, H-3), 3.84 (3H, s, OMe); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.0 (C-4), 164.0 (C-2), 158.6 (C-6), 133.7 (C-12a), 132.7 (C-9), 132.6 (C-8), 131.4 (C-16a), 129.8 (C-12), 128.6 (C-13), 126.7 (C-15), 126.2 (C-14), 125.4 (C-11), 124.1 (C-10), 123.6 (C-16), 121.3 (C-7), 101.6 (C-5), 89.0 (C-3), 55.9 (OMe); EIMS *m/z* 278 $[M]^+$; HREIMS *m/z* 278.09419 (calcd for $C_{18}H_{14}O_3$ 278.09429); Anal. Calcd for $C_{18}H_{14}O_3.1/5H_2O$: C, 76.69; H, 5.15. Found: C, 76.57; H, 5.21.

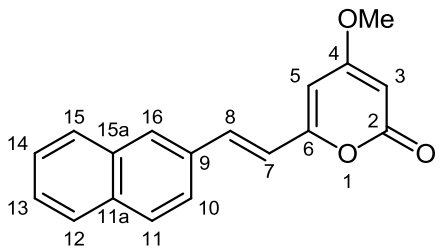
6,6'-(1E,1'E)-2,2'-(1,4-phenylene)bis(ethene-2,1-diyl)bis(4-methoxy-2H-pyran-2-one) (**3.54**):



Dipyrone (**3.54**) was prepared and purified under standard conditions from pyrone **3.40** (202 mg, 1.4 mmol) and terephthaldicarboxaldehyde (98 mg, 0.73 mmol). Recrystallisation from methanol afforded **3.54** as a yellow solid (28 mg, 10 %). The application of light exclusion methodology with pyrone **3.40** (200 mg, 1.4 mmol) and terephthaldicarboxaldehyde (94.6 mg, 0.7 mmol) gave an increased yield (72.4 mg, 27%).

Mp 330 ºC (decomp); TLC R_f (3% MeOH:CH_2Cl_2) 0.15; IR (smear) ν_{max} 3078, 1726, 1640, 1610, 1556, 1453, 1409, 1256, 1154, 950, 813 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.50 (4H, s, H-10), 7.48 (2H, d, *J* = 14.8 Hz, H-8), 6.60 (2H, d, *J* = 16.0 Hz, H-7), 5.96 (2H, d, *J* = 2.0 Hz, H-5), 5.50 (2H, d, *J* = 2.0 Hz, H-3), 3.83 (6H, s, OMe); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.0 (C-4), 163.9 (C-2), 158.4 (C-6), 136.3 (C-9), 134.8 (C-8), 128.0 (C-10), 119.3 (C-7), 101.8 (C-5), 89.1 (C-3), 56.0 (OMe); FABMS *m/z* 379 $[M+H]^+$; HRFABMS *m/z* 379.11877 (calcd for $C_{22}H_{19}O_6$ 379.11816); Anal. Calcd for $C_{22}H_{18}O_6.1/5CH_2Cl_2$: C, 67.44; H, 4.69. Found: C, 67.17; H, 4.78.

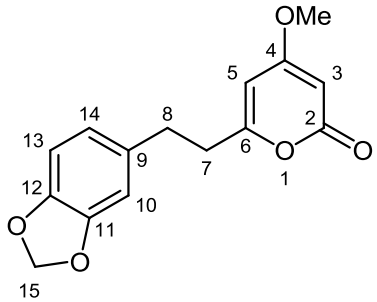
4-Methoxy-6-[2-(2-naphthyl)ethenyl]-2H-pyran-2-one (**3.55**):



Pyrone (**3.55**) was prepared and purified under standard conditions from pyrone **3.40** (200 mg, 1.4 mmol) and 2-napthaldehyde (268 mg, 1.7 mmol). Repeated recrystallisation from methanol afforded **3.55** as a yellow crystalline solid (38 mg, 9.6 %). The application of light exclusion methodology with the pyrone (201 mg, 1.4 mmol) and 2-napthaldehyde (269 mg, 1.7 mmol) gave **3.55** in higher yield (138 mg, 35%).

Mp 201-202 ºC; TLC R_f (3% MeOH:CH_2Cl_2) 0.56; IR (smear) ν_{max} 3081, 1709, 1638, 1549, 1448, 1408, 1251, 1143, 957, 813, 754 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.87 (1H, s, H-16), 7.82 (1H, m, H-15), 7.81 (1H, d, *J* = 8.5 Hz, H-11), 7.80 (1H, m, H-12), 7.64 (1H, d, *J* = 16.2 Hz, H-8), 7.63 (1H, dd, *J* = 8.8, 1.7 Hz, H-10), 7.49 (1H, m, H-14), 7.48 (1H, m, H-13), 6.68 (1H, d, *J* = 15.9 Hz, H-7), 5.96 (1H, d, *J* = 2.2 Hz, H-5), 5.49 (1H, d, *J* = 2.2 Hz, H-3), 3.81 (3H, s, OMe), ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.0 (C-4), 164.0 (C-2), 158.7 (C-6), 135.8 (C-8), 133.8 (C-11a), 133.4 (C-15a), 132.7 (C-9), 128.8 (C-16), 128.6 (C-11), 128.4 (C-15), 127.7 (C-12), 126.9 (C-13), 126.6 (C-14), 123.2 (C-10), 118.8 (C-7), 101.3 (C-5), 88.8 (C-3), 55.9 (OMe); EIMS *m/z* 278 $[M]^+$; HREIMS *m/z* 278.09445 (calcd for $C_{18}H_{14}O_3$ 278.09429); Anal. Calcd for $C_{18}H_{14}O_3.1/10H_2O$: C, 77.18; H, 5.11. Found: C, 77.31; H, 5.06.

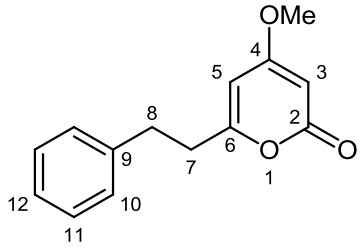
6-[2-(1,3-Benzodioxol-5-yl)ethyl]-4-methoxy-2H-pyran-2-one (**3.33**):



A mixture of **3.36** (17.9 mg, 0.66 mmol) and Pd/C (10%, 4.8 mg) was stirred under hydrogen for 2 hrs in methanol:chloroform (1:1), at which time the resulting suspension was filtered and the filtrate dried in vacuo. The product was recrystallised from methanol:water to afford a white crystalline material **3.33** (13.0 mg, 72%).

Mp 161-162 ºC (lit.[138] 158-160 ºC); TLC R_f (3% MeOH:CH_2Cl_2) 0.39; IR (smear) ν_{max} 3108, 2927, 1705, 1645, 1567, 1489, 1414, 1239, 1030, 920, 824, 809 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.71 (1H, d, *J* = 7.9 Hz, H-13), 6.64 (1H, s, H-10), 6.60 (1H, d, *J* = 7.8 Hz, H-14), 5.91 (2H, s, H-15), 5.70 (1H, d, *J* = 2.3 Hz, H-5), 5.40 (1H, d, *J* = 2.1 Hz, H-3), 3.77 (3H, s, OMe), 2.88 (2H, t, *J* = 7.2 Hz, H-8), 2.68 (2H, t, *J* = 7.6 Hz, H-7); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.1 (C-4), 164.9 (C-2), 164.2 (C-6), 147.7 (C-11), 146.1 (C-12), 133.6 (C-9), 121.2 (C-14), 108.7 (C-10), 108.3 (C-13), 100.9 (C-15), 100.3 (C-5), 87.7 (C-3), 55.8 (OMe), 35.7 (C-7), 32.6 (C-8); EIMS *m/z* 274 $[M]^+$; HREIMS *m/z* 274.08407 (calcd for $C_{15}H_{14}O_5$ 274.08412); Anal. Calcd for $C_{15}H_{14}O_5$: C, 65.69; H, 5.15. Found: C, 65.76; H, 5.23. Crystal structure.

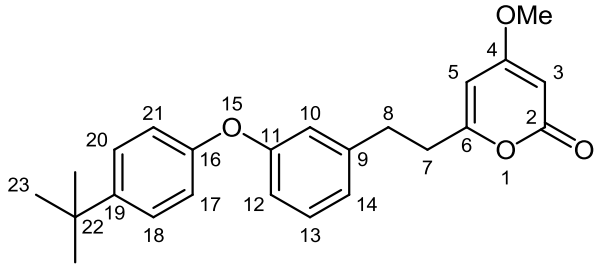
7,8-Dihydro-4-methoxy-6-styryl-2H-pyran-2-one (**3.56**):



A mixture of **3.39** (24.9 mg, 0.11 mmol) and Pd/C (10%, 6.8 mg) was stirred under hydrogen for 2 hrs in methanol:chloroform (1:1), at which time the resulting suspension was filtered and the filtrate dried in vacuo. The product was recrystallised from methanol:water to yield a white crystalline solid **3.56** (16.9 mg, 67%).

Mp 99-100 ºC (lit.[139] 98.5 °C); TLC R_f (3% MeOH:CH_2Cl_2) 0.59; IR (smear) ν_{max} 3077, 1727, 1700, 1651, 1569, 1453, 1426, 1266, 1233, 1134, 1033, 860, 843, 814, 745, 703 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.28 (2H, t, *J* = 7.1 Hz, H-11), 7.21 (1H, t, *J* = 7.2 Hz, H-12), 7.16 (2H, d, *J* = 7.1 Hz, H-10), 5.71 (1H, d, *J* = 2.4 Hz, H-5), 5.40 (1H, d, *J* = 2.0 Hz, H-3), 3.77 (3H, s, OMe), 2.96 (2H, t, *J* = 7.2 Hz, H-8), 2.74 (2H, t, *J* = 7.2 Hz, H-7); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.1 (C-4), 164.9 (C-2), 164.3 (C-6), 139.8 (C-9), 128.6 (C-11), 128.3 (C-10), 126.4 (C-12), 100.3 (C-5), 87.7 (C-3), 55.8 (OMe), 35.4 (C-7), 32.8 (C-8); EIMS *m/z* 230 $[M]^+$; HREIMS *m/z* 230.09386 (calcd for $C_{14}H_{14}O_3$ 230.09429); Anal. Calcd for $C_{14}H_{14}O_3$: C, 73.03; H, 6.13. Found: C, 73.30; H, 6.11.

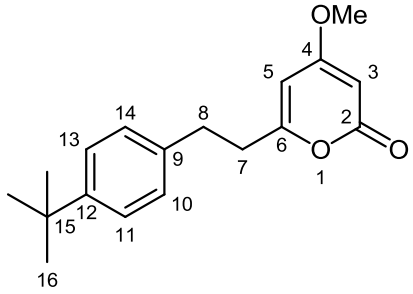
4-Methoxy-6-[2-(3-(4-tert-butylphenoxy)phenyl)ethyl]-2H-pyran-2-one (**3.57**):



A mixture of **3.44** (21.2 mg, 0.056 mmol) and Pd/C (10%, 3.8 mg) was stirred under hydrogen for 2 hrs in methanol:chloroform (1:1), at which time the resulting suspension was filtered and the filtrate dried in vacuo. The product was purified by flash column chromatography (silica gel, 0 to 0.75% methanol in dichloromethane) to afford a colourless oil **3.57** (19.6 mg, 92%).

TLC R_f (3% MeOH:CH_2Cl_2) 0.49; IR (smear) ν_{max} 2960, 2905, 2864, 1721, 1650, 1568, 1508, 1485, 1454, 1410, 1247, 1220, 1176, 1138, 1038, 1014, 941, 816, 787, 699; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33 (2H, d, *J* = 8.7 Hz, H-18,20), 7.22 (1H, t, *J* = 7.8 Hz, H-13), 6.90 (2H, d, *J* = 8.7 Hz, H-17,21), 6.81 (1H, m, H-10), 6.83 (1H, m, H-12), 6.87 (1H, m, H-14), 5.71 (1H, d, *J* = 1.9 Hz, H-5), 5.40 (1H, d, *J* = 2.1 Hz, H-3), 3.77 (3H, s, OMe), 2.93 (2H, m, H-8), 2.72 (2H, m, H-7), 1.32 (9H, s, H-23);^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.1 (C-4), 164.8 (C-2), 164.2 (C-6), 157.8 (C-11), 154.5 (C-16), 146.2 (C-19), 141.7 (C-9), 129.8 (C-13), 126.5 (C-18,20), 122.9 (C-14), 118.4 (C-17,21), 118.3 (C-10), 116.6 (C-12), 100.3 (C-5), 87.7 (C-3), 55.8 (OMe), 35.2 (C-7), 34.3 (C-22), 32.7 (C-8), 31.5 (C-23); (+)-ESIMS *m/z* 379 $[M+H]^+$, (+)-HRESIMS *m/z* 379.1906 (calcd for $C_{24}H_{27}O_4$ 379.1904).

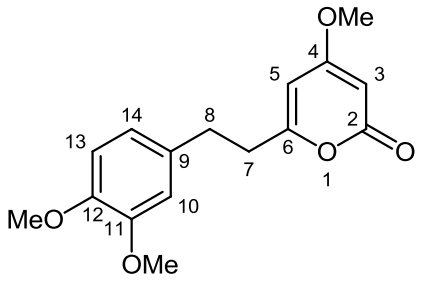
4-Methoxy-6-[2-(4-tert-butylphenyl)ethyl] -2H-pyran-2-one (**3.58**):



A mixture of **3.46** (19.3 mg, 0.068 mmol) and Pd/C (10%, 5.2 mg) was stirred under hydrogen for 2 hrs in methanol:chloroform (1:1), at which time the resulting suspension was filtered and the filtrate dried in vacuo. The product was purified by flash column chromatography (silica gel, 0 to 0.75% methanol in dichloromethane) to afford a white solid material **3.58** (17.4 mg, 90%).

Mp 77-79 ºC; TLC R_f (3% MeOH:CH_2Cl_2) 0.42; IR (smear) ν_{max} 3085, 2959, 2906, 2864, 1713, 1651, 1567, 1451, 1411, 1248, 1138, 1031, 1008, 936, 871, 849, 822 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.31 (2H, d, *J* = 8.3 Hz, H-11,13), 7.11 (2H, d, *J* = 8.2 Hz, H-10,14), 5.75 (1H, d, *J* = 1.9 Hz, H-5), 5.42 (1H, d, *J* = 2.1 Hz, H-3), 3.77 (3H, s, OMe), 2.92 (2H, m, H-8), 2.73 (2H, m, H-7), 1.30 (9H, s, H-16); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.2 (C-4), 165.0 (C-2), 164.6 (C-6), 149.3 (C-12), 136.8 (C-9), 127.9 (C-10,14), 125.5 (C-11,13), 100.1 (C-5), 87.7 (C-3), 55.8 (OMe), 35.4 (C-7), 34.4 (C-15), 32.3 (C-8), 31.3 (C-16); (+)-ESIMS *m/z* 287 $[M+H]^+$, (+)-HRESIMS *m/z* 287.1639 (calcd for $C_{18}H_{23}O_3$ 287.1642).

6-[2-(3,4-Dimethoxyphenyl)ethyl]-4-methoxy-2H-pyran-2-one (**3.59**):

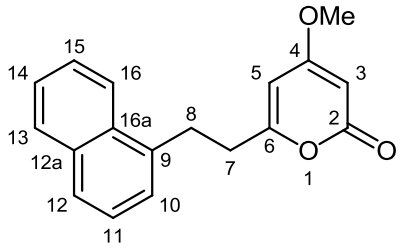


A mixture of **3.47** (20.8 mg, 0.072 mmol) and Pd/C (10%, 3.9 mg) was stirred under hydrogen for 2 hrs in methanol:chloroform (1:1), at which time the resulting suspension was filtered and the filtrate dried in vacuo. The product was purified by flash column chromatography (silica gel, 0 to 1% methanol in dichloromethane) to afford a white solid material **3.59** (20.1 mg, 96%).

Mp 71-73 ºC; TLC R_f (3% MeOH:CH_2Cl_2) 0.18; IR (smear) ν_{max} 3002, 2947, 2842, 1709, 1651, 1569, 1518, 1453, 1407, 1257, 1241, 1163, 1136, 1032, 1022, 944, 864, 819, 809 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.77 (1H, d, *J* = 8.1 Hz, H-13), 6.69 (1H, dd, *J* = 8.1, 2.0 Hz, H-14), 6.66 (1H, d, *J* = 1.9 Hz, H-10), 5.69 (1H, d, *J* = 2.2

Hz, H-5), 5.40 (1H, d, *J* = 2.3 Hz, H-3), 3.83 (3H, s, OMe-11,12), 3.82 (3H, s, OMe-11,12), 3.75 (3H, s, OMe-4), 2.89 (2H, m, H-8), 2.70 (2H, m, H-7); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.2 (C-4), 165.1 (C-2), 164.3 (C-6), 148.8 (C-11), 147.5 (C-12), 132.4 (C-9), 120.1 (C-14), 111.6 (C-10), 111.3 (C-13), 100.4 (C-5), 87.6 (C-3), 55.8 (OMe), 55.8 (OMe), 35.7 (C-7), 32.4 (C-8), (+)-ESIMS *m/z* 291 $[M+H]^+$, (+)-HRESIMS *m/z* 291.1234 (calcd for $C_{16}H_{19}O_5$ 291.1227).

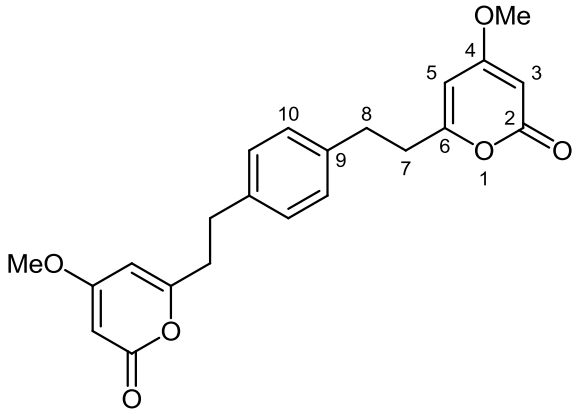
4-Methoxy-6-[2-(1-naphthyl)ethyl]-2H-pyran-2-one (**3.60**):



A mixture of **3.53** (21.1 mg, 0.076 mmol) and Pd/C (10%, 4.7 mg) was stirred under hydrogen for 6 hrs in methanol:chloroform (1:1), at which time the resulting suspension was filtered and the filtrate dried in vacuo. The product was purified by flash column chromatography (silica gel, 0 to 1% methanol in dichloromethane) to afford a white solid material **3.60** (16.0 mg, 75%).

Mp 75-77 ºC; TLC R_f (3% MeOH:CH_2Cl_2) 0.40; IR (smear) ν_{max} 3078, 3018, 2952, 1714, 1647, 1565, 1465, 1449, 1411, 1257, 1238, 1138, 1035, 940, 871, 847, 835, 811, 796, 777, 762 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.02 (1H, d, *J* = 8.4, H-16), 7.86 (1H, d, *J* = 7.8, H-13), 7.73 (1H, d, *J* = 8.2, H-12), 7.53 (1H, m, H-15), 7.48 (1H, m, H-14), 7.38 (1H, t, *J* = 7.6, H-11), 7.30 (1H, d, *J* = 6.7, H-10), 5.71 (1H, d, *J* = 2.2, H-5), 5.43 (1H, d, *J* = 2.1, H-3), 3.76 (3H, s, OMe), 3.43 (2H, m, H-8), 2.86 (2H, m, H-7); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.1 (C-4), 164.9 (C-2), 164.4 (C-6), 135.9 (C-9), 133.9 (C-12a), 131.5 (C-16a), 128.9 (C-13), 127.3 (C-12), 126.2 (C-10,15), 125.6 (C-11,14), 125.5 (C-11,14), 123.2 (C-16), 100.3 (C-5), 87.8 (C-3), 55.8 (OMe), 34.8 (C-7), 30.2 (C-8); (+)-ESIMS *m/z* 281 $[M+H]^+$, (+)-HRESIMS *m/z* 281.1174 (calcd for $C_{18}H_{17}O_3$ 281.1172).

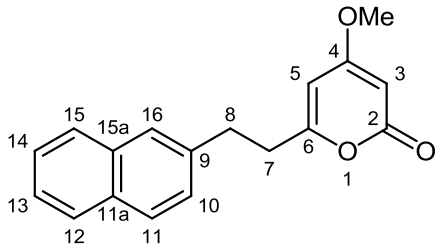
6,6'-(2,2'-(1,4-Phenylene)bis(ethane-2,1-diyl))bis(4-methoxy-2H-pyran-2-one) (**3.61**):



A mixture of **3.54** (19.7 mg, 0.052 mmol) and Pd/C (10%, 4.9 mg) was stirred under hydrogen for 20 hrs in methanol:chloroform (1:1), at which time the resulting suspension was filtered and the filtrate dried in vacuo. The product was purified by flash column chromatography (silica gel, 0 to 1% methanol in dichloromethane) to afford a white solid material **3.61** (9.8 mg, 49%).

Mp 196-198 ºC; TLC R_f (3% MeOH:CH_2Cl_2) 0.06; IR (smear) ν_{max} 3079, 2953, 2923, 2863, 1710, 1651, 1566, 1450, 1413, 1250, 1143, 1030, 1024, 1001, 937, 871, 853, 836, 816, 784, 738, 690, 648, 631 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.08 (4H, s, H-10), 5.70 (2H, d, *J* = 2.0 Hz, H-5), 5.40 (2H, d, *J* = 2.2 Hz, H-3), 3.77 (6H, s, OMe), 2.92 (4H, m, H-8), 2.71 (4H, m, H-7); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.1 (C-4), 164.8 (C-2), 164.3 (C-6), 138.0 (C-9), 128.5 (C-10), 100.2 (C-5), 87.7 (C-3), 55.8 (OMe), 35.4 (C-7), 32.4 (C-8); (+)-ESIMS *m/z* 383 $[M+H]^+$, (+)-HRESIMS *m/z* 383.1496 (calcd for $C_{22}H_{23}O_6$ 383.1489).

4-Methoxy-6-[2-(2-naphthyl)ethyl]-2H-pyran-2-one (**3.62**):

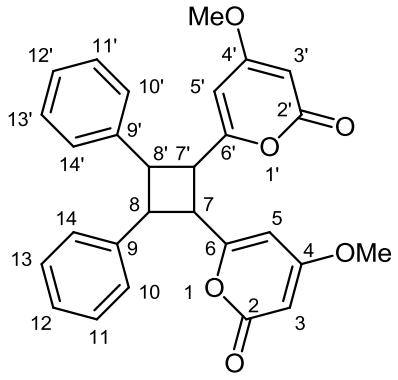


A mixture of **3.55** (19.4 mg, 0.070 mmol) and Pd/C (10%, 3.8 mg) was stirred under hydrogen for 6 hrs in methanol:chloroform (1:1), at which time the resulting

suspension was filtered and the filtrate dried in vacuo. ^{1}H NMR analysis of the crude product indicated incomplete reaction, so the solid was further stirred under hydrogen in methanol:chloroform (1:1) for 5 hrs and worked up again. The product was purified by flash column chromatography (silica gel, 0 to 0.5% methanol in dichloromethane) to afford a white solid material **3.62** (16.3 mg, 84%).

Mp 72-74 ºC; TLC R_f (3% MeOH:CH_2Cl_2) 0.42; IR (smear) ν_{max} 3082, 3056, 2947, 2925, 2848, 1722, 1649, 1564, 1455, 1412, 1264, 1250, 1142, 1035, 1018, 936, 860, 849, 819, 803, 759, 733 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.77 (3H, m, H-11,12,15), 7.60 (1H, s, H-16), 7.43 (2H, m, H-13,14), 7.29 (1H, dd, *J* = 8.4, 1.6 Hz, H-10), 5.72 (1H, d, *J* = 2.1 Hz, H-5), 5.41 (1H, d, *J* = 2.1 Hz, H-3), 3.74 (3H, s, OMe), 3.12 (2H, m, H-8), 2.83 (2H, m, H-7); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.2 (C-4), 165.1 (C-2), 164.2 (C-6), 137.2 (C-9), 133.5 (C-15a), 132.1 (C-11a), 128.2 (C-11,12,15), 127.6 (C-11,12,15), 127.5 (C-11,12,15), 126.7 (C-10), 126.6 (C-16), 126.1 (C-13,14), 125.5 (C-13,14), 100.4 (C-5), 87.7 (C-3), 55.8 (OMe), 35.3 (C-7), 32.9 (C-8); (+)-ESIMS *m/z* 281 $[M+H]^+$, (+)-HRESIMS *m/z* 281.1172 (calcd for $C_{18}H_{17}O_3$ 281.1172).

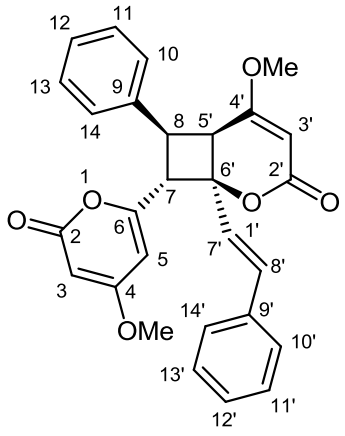
6,6'-(3,4-Diphenylcyclobutane-1,2-diyl)bis(4-methoxy-2H-pyran-2-one) (**3.63**):



Pyrone **3.39** (2 samples totaling 43.0 mg) was suspended, with stirring, in water and exposed to a sunlamp (15 cm from vial) for three 5 hr periods. Upon noting the loss of starting material in the ^{1}H NMR, the resulting solid was purified by repeated flash column chromatography (silica gel, 0 to 5% methanol in dichloromethane) to give symmetric dimer **3.63** as an amorphous colourless solid (4.2 mg, 10 %) and the asymmetric dimer **3.64** as an amorphous colourless solid (8.0 mg, 19 %).

Mp 82-84 ºC (lit.[113] 103-105 ºC); TLC R_f (3% MeOH:CH_2Cl_2) 0.24; IR (smear) ν_{max} 2918, 2850, 1714, 1646, 1563, 1454, 1409, 1239, 1153, 1033, 991, 963, 810, 729, 696 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.12 (4H, m, H-11,13,11',13'), 7.06 (2H, m, H-12,12'), 6.95 (4H, m, H-10,14,10',14'), 5.99 (2H, d, *J* = 2.3 Hz, H-5,5'), 5.36 (2H, d, *J* = 2.3 Hz, H-3,3'), 4.50 (2H, m, H-8,8'), 4.11 (2H, m, H-7,7'), 3.75 (6H, s, OMe-4,4'); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.0 (C-4,4'), 164.3 (C-2,2'), 162.4 (C-6,6'), 137.9 (C-9,9'), 128.2 (C-11,13,11',13'), 127.7 (C-10,14,10',14'), 126.7 (C-12,12'), 101.5 (C-5,5'), 88.0 (C-3,3'), 55.9 (OMe-4,4'), 44.9 (C-8,8'), 44.0 (C-7,7'); (+)-ESIMS *m/z* 457 $[M+H]^+$, (+)-HRESIMS *m/z* 457.1650 (calcd for $C_{28}H_{25}O_6$ 457.1646); (+)-HRESIMSMS (parent *m/z* 457) Fragment *m/z* 277.0701 (calcd for $C_{14}H_{13}O_6$ 277.0707), 229.0853 (calcd for $C_{14}H_{13}O_3$ 229.0859), 179.0324 (calcd for $C_9H_7O_4$ 179.0339).

Aniba-dimer-A (**3.64**):

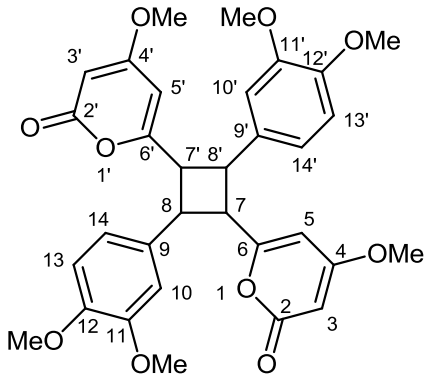


As isolated stated above.

Mp 178-180 ºC (lit.[140] 178-179 ºC); TLC R_f (3% MeOH:CH_2Cl_2) 0.20; IR (smear) ν_{max} 3078, 3025, 2942, 1704, 1647, 1623, 1567, 1456, 1442, 1416, 1396, 1258, 1244, 1209, 1141, 978, 961, 817, 792, 764, 741, 731, 700 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.41 (2H, m, H-10',14'), 7.29 (8H, m, H-10,11,12,13,14,11',12',13'), 6.94 (1H, d, *J* = 15.9 Hz, H-8'), 6.59 (1H, d, *J* = 15.8 Hz, H-7'), 5.91 (1H, d, *J* = 2.2 Hz, H-5), 5.34 (1H, d, *J* = 2.1 Hz, H-3), 5.28 (1H, s, H-3'), 4.35 (1H, t, *J* = 10.3 Hz, H-8), 4.16 (1H, d, *J* = 11.1 Hz, H-7), 3.71 (3H, s, OMe-4), 3.59 (1H, d, *J* = 10.1 Hz, H-5'), 3.27 (3H,

s, OMe-4'); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.5 (C-4), 169.9 (C-4'), 164.6, 163.9 (C-2,2'), 158.6 (C-6), 135.9 (C-9'), 135.6 (C-9), 131.5 (C-8'), 128.7, 128.5, 128.3, 127.8 (C-11,12,13,11',12',13'), 127.5 (C-10,14), 126.9 (C-10',14'), 124.4 (C-7'), 102.7 (C-5), 91.8 (C-3'), 88.7 (C-3), 79.4 (C-6'), 55.9 (OMe-4), 55.4 (OMe-4'), 54.5 (C-7), 45.7 (C-5'), 39.2 (C-8); (+)-ESIMS *m/z* 479 $[M+Na]^+$, (+)-HRESIMS *m/z* 479.1453 (calcd for $C_{28}H_{24}O_6Na$ 479.1465).

6,6'-(2,4-Bis(3,4-dimethoxyphenyl)cyclobutane-1,3-diyl)bis(4-methoxy-2H-pyran-2-one) (**3.66**):

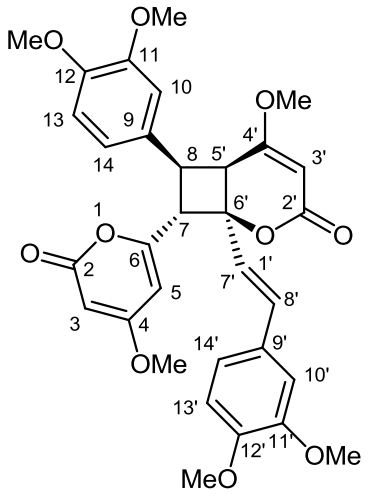


Pyrone **3.47** (19.9 mg) was suspended, with stirring, in water and exposed to a sunlamp (15 cm from vial) for three 5 hr periods. Upon noting the loss of starting material in the ^{1}H NMR, the resulting solid was purified by repeated flash column chromatography (silica gel, 0 to 3% methanol in dichloromethane) to give symmetric dimer **3.66** as an amorphous colourless solid (4.1 mg, 14 %) and the asymmetric dimer **3.67** as an amorphous yellow solid (6.9 mg, 35 %).

Mp 86-88 ºC (lit.[118] 112-114 ºC); TLC R_f (3% MeOH:CH_2Cl_2) 0.09; IR (smear) ν_{max} 2918, 2849, 1706, 1641, 1563, 1515, 1454, 1409, 1249, 1142, 1023, 968, 801, 764 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.85 (2H, dd, *J* = 8.2, 2.0 Hz, H-14,14'), 6.79 (2H, d, *J* = 7.9 Hz, H-13,13'), 6.77 (2H, br, H-10,10'), 5.69 (2H, d, *J* = 2.2 Hz, H-5,5'), 5.22 (2H, d, *J* = 2.2 Hz, H-3,3'), 4.34 (2H, m, H-8,8'), 4.19 (2H, m, H-7,7'), 3.85 (6H, s, OMe-11,11'), 3.83 (6H, s, OMe-12,12'), 3.68 (6H, s, OMe-4,4'); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.6 (C-4,4'), 164.0 (C-2,2'), 162.9 (C-6,6'), 148.9 (C-11,11'), 148.2 (C-12,12'), 129.8 (C-9,9'), 119.4 (C-14,14'), 111.04, 110.96 (C-10,13,10',13'), 101.3 (C-5,5'), 87.8 (C-3,3'), 56.0 (OMe-11,11'), 55.82 (OMe-12,12'), 55.76 (OMe-4,4'), 45.5

(C-7,7'), 43.5 (C-8,8'); (+)-ESIMS *m/z* 577 $[M+H]^+$, (+)-HRESIMS *m/z* 577.2073 (calcd for $C_{32}H_{33}O_{10}$ 577.2068); (+)-HRESIMSMS (parent *m/z* 577) Fragment *m/z* 289.1055 (calcd for $C_{16}H_{17}O_5$ 289.1071), 191.0689 (calcd for $C_{11}H_{11}O_3$ 191.0703).

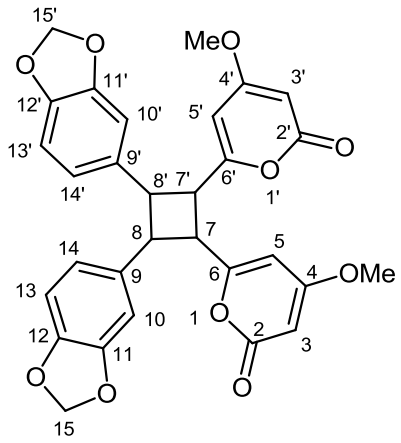
11,12,11',12'-Tetramethoxy-aniba-dimer-A (**3.67**):



As stated above.

Mp 99-100 ºC (lit.[118] 195-197 ºC); TLC R_f (3% MeOH:CH_2Cl_2) 0.05; IR (smear) ν_{max} 2938, 2838, 1705, 1645, 1623, 1564, 1515, 1454, 1411, 1390, 1250, 1208, 1139, 1023, 991, 961, 813, 761, 726 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.99 (1H, d, *J* = 1.9 Hz, H-10'), 6.93 (1H, dd, *J* = 8.3, 1.9 Hz, H-14'), 6.86 (1H, d, *J* = 15.8 Hz, H-8'), 6.79 (4H, m, H-10,13,14,13'), 6.48 (1H, d, *J* = 15.7 Hz, H-7'), 5.90 (1H, d, *J* = 2.2 Hz, H-5), 5.33 (1H, d, *J* = 2.1 Hz, H-3), 5.29 (1H, s, *J* = 5.3 Hz, H-3'), 4.32 (1H, t, *J* = 10.3 Hz, H-8), 4.06 (1H, d, *J* = 10.8 Hz, H-7), 3.93 (3H, s), 3.88 (3H, s), 3.87 (3H, s), 3.86 (3H, s, OMe-11,12,11',12'), 3.71 (3H, s, OMe-4), 3.57 (1H, d, *J* = 9.8 Hz, H-5'), 3.33 (3H, s, OMe-4'); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.6 (C-4), 170.2 (C-4'), 164.7, 164.0 (C-2, 2'), 158.6 (C-6), 149.3, 149.2, 148.9, 148.7 (C-11,12,11',12'), 130.9 (C-6'), 129.0 (C-9'), 128.3 (C-9), 122.5 (C-7'), 120.3 (C-14'), 119.1, 111.3, 111.1, 110.8 (C-10,13,14,13'), 108.8 (C-10'), 102.8 (C-5), 91.6 (C-3'), 88.6 (C-3), 79.4 (C-6'), 56.0, 55.9 (OMe-4,11,12,11',12'), 55.6 (OMe-4'), 55.3 (C-7), 45.9 (C-5'), 39.1 (C-8); (+)-ESIMS *m/z* 577 $[M+H]^+$, (+)-HRESIMS *m/z* 577.2056 (calcd for $C_{32}H_{33}O_{10}$ 577.2068).

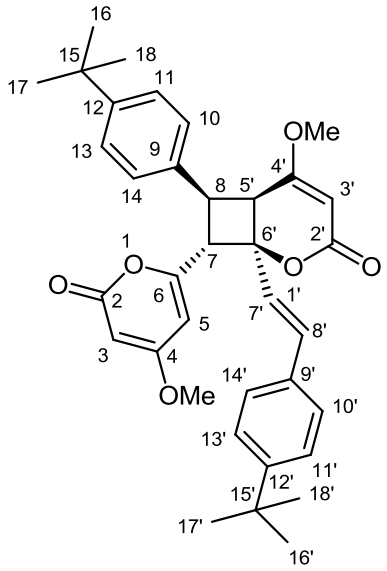
6,6'-(3,4-Di(benzo[d][1,3]dioxol-5-yl)cyclobutane-1,2-diyl)bis(4-methoxy-2H-pyran-2-one) (**3.68**):



Pyrone **3.36** (19.3 mg) was suspended, with stirring, in water and exposed to a sunlamp (15 cm from vial) for three 5 hr periods. The resulting solid was purified by repeated flash column chromatography (silica gel, 0 to 2% methanol in dichloromethane) to give symmetric dimer **3.68** as an amorphous colourless solid (2.0 mg, 10 %) and starting material **3.36** as an amorphous yellow solid (5.6 mg, 29 %).

Mp 101-103 ºC; TLC R_f (3% MeOH:CH_2Cl_2) 0.14; IR (smear) ν_{max} 2905, 1706, 1646, 1561, 1504, 1490, 1445, 1408, 1237, 1155, 1034, 931, 812, 727 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.62 (2H, d, *J* = 8.0 Hz, H-13,13'), 6.46 (2H, dd, *J* = 8.0, 1.7 Hz, H-14,14'), 6.43 (2H, d, *J* = 1.6 Hz, H-10,10'), 5.96 (2H, d, *J* = 2.1 Hz, H-5,5'), 5.86 (4H, br s, H-15,15'), 5.35 (2H, d, *J* = 2.1 Hz, H-3,3'), 4.35 (2H, pseudo d, *J* = 6.6 Hz, H-8,8'), 3.96 (2H, pseudo d, *J* = 6.5 Hz, H-7,7'), 3.75 (6H, s, OMe-4,4'); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.0 (C-4,4'), 164.2 (C-2,2'), 162.2 (C-6,6'), 147.7 (C-11,11'), 146.3 (C-12,12'), 131.8 (C-9,9'), 120.8 (C-14,14'), 108.1 (C-10,10'), 101.4 (C-5,5'), 100.9 (C-15,15'), 88.1 (C-3,3'), 55.9 (OMe-4,4'), 44.8 (C-8,8'), 44.4 (C-7,7'); (+)-ESIMS *m/z* 545 $[M+H]^+$, (+)-HRESIMS *m/z* 545.1435 (calcd for $C_{30}H_{25}O_{10}$ 557.1959), (+)-HRESIMSMS (parent *m/z* 545) Fragment *m/z* 277.0690 (calcd for $C_{14}H_{13}O_6$ 277.0707), 179.0317 (calcd for $C_9H_7O_4$ 179.0339).

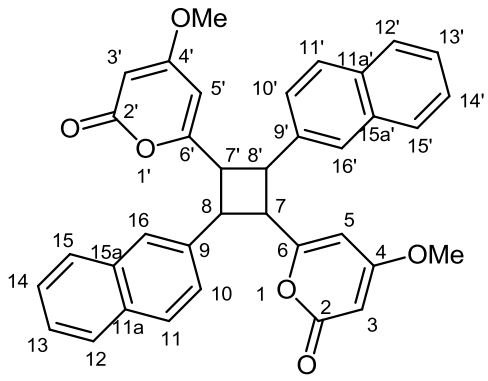
12,12'-Di-tert-butyl-aniba-dimer-A (**3.69**):



Pyrone **3.46** (20.6 mg, 0.036 mmol) was suspended, with stirring, in water and exposed to a sunlamp (15 cm from vial) for two 5 hr periods. Upon noting the loss of starting material in the ^{1}H NMR spectrum, the resulting solid was purified by repeated flash column chromatography (silica gel, 0 to 3% methanol in dichloromethane) to give asymmetric dimer **3.69** as an amorphous colourless solid (9.5 mg, 46 %).

Mp 130-131 ºC; TLC R_f (3% MeOH:CH_2Cl_2) 0.26; IR (smear) ν_{max} 2960, 2905, 2865, 1713, 1646, 1626, 1567, 1455, 1409, 1390, 1244, 1207, 1143, 968, 817 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35 (4H, s, H-10',11',13',14'), 7.34 (2H, d, *J* = 9.9 Hz, H-11,13), 7.16 (2H, d, *J* = 8.3 Hz, H-10,14), 6.91 (1H, d, *J* = 15.9 Hz, H-8'), 6.55 (1H, d, *J* = 15.9 Hz, H-7'), 5.89 (1H, d, *J* = 2.1 Hz, H-5), 5.33 (1H, d, *J* = 2.1 Hz, H-3), 5.27 (1H, s, H-3'), 4.33 (1H, t, *J* = 10.3 Hz, H-8), 4.14 (1H, d, *J* = 11.0 Hz, H-7), 3.70 (3H, s, OMe-4), 3.53 (1H, d, *J* = 9.6 Hz, H-5'), 3.24 (3H, s, OMe-4'), 1.30 (9H, s), 1.29 (9H, s, H-16,16'); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.5 (C-4), 170.1 (C-4'), 164.8, 164.0 (C-2,2'), 158.8 (C-6), 151.4 (C-12'), 150.9 (C-12), 133.1 (C-9'), 132.6 (C-9), 131.1 (C-8'), 127.2 (C-10,14), 126.6 (C-10',14'), 125.7 (C-11',13'), 125.3 (C-11,13), 123.6 (C-7'), 102.6 (C-5), 91.7 (C-3'), 88.7 (C-3), 79.5 (C-6'), 55.8 (OMe-4), 55.2 (OMe-4'), 54.5 (C-7), 45.8 (C-5'), 38.8 (C-8), 34.6, 34.5 (C-15,15'), 31.3, 31.2 (C-16,16'); (+)-ESIMS *m/z* 569 [M+H]$^{+}$, (+)-HRESIMS *m/z* 569.2877 (calcd for $C_{36}H_{41}O_6$ 569.2898).

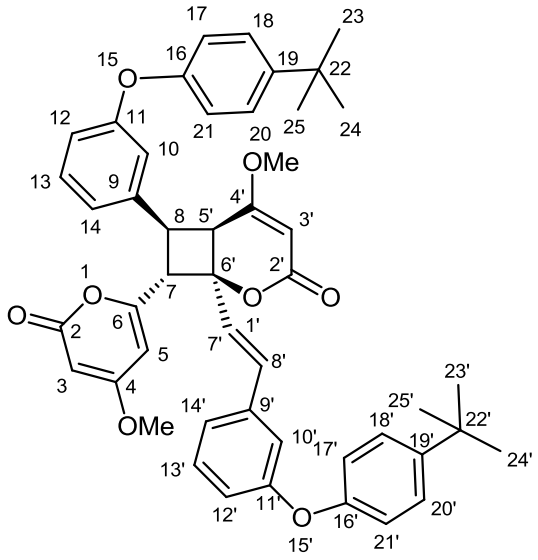
6,6'-(2,4-Di(naphthalen-2-yl)cyclobutane-1,3-diyl)bis(4-methoxy-2H-pyran-2-one) (**3.70**):



Pyrone **3.55** (21.3 mg) was suspended, with stirring, in water and exposed to a sunlamp (15 cm from vial) for two 5 hr periods. Upon noting the loss of starting material in the ^{1}H NMR, the resulting solid was purified by flash column chromatography (silica gel, 0 to 2% methanol in dichloromethane) to give an amorphous colourless solid **3.70** (4.12 mg, 19 %).

Mp 130-131 ºC; TLC R_f (3% MeOH:CH_2Cl_2) 0.20; IR (smear) ν_{max} 3055, 2946, 1714, 1644, 1564, 1454, 1410, 1246, 1146, 1126, 809, 747, 732 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.80 (8H, m, H-nap), 7.45 (6H, m, H-nap), 5.77 (2H, d, *J* = 2.2 Hz, H-5,5'), 5.13 (2H, d, *J* = 2.1 Hz, H-3,3'), 4.67 (2H, m, H-8,8'), 4.48 (2H, m, H-7,7'), 3.60 (6H, s, OMe-4,4'); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.4 (C-4,4'), 163.9 (C-2,2'), 162.6 (C-6,6'), 134.9 (C-9,9'), 133.3, 132.5 (C-11a,15a,11a',15a'), 128.3, 127.9, 127.6, 126.2, 126.1, 125.9, 125.8 (C-10,11,12,13,14,15,16,10',11',12',13',14',15',16'), 101.5 (C-5,5'), 87.9 (C-3,3'), 55.7 (OMe-4,4'), 45.3 (C-7,7'), 44.0 (C-8,8'); (+)-ESIMS *m/z* 557 $[M+H]^+$, (+)-HRESIMS *m/z* 557.1963 (calcd for $C_{36}H_{29}O_6$ 557.1959); (+)-HRESIMSMS (parent *m/z* 557) Fragment *m/z* 279.1005 (calcd for $C_{18}H_{15}O_3$ 279.1016), 181.0648 (calcd for $C_{13}H_9O$ 181.0648).

11,11' Di (4-tert-butyl-phenoxy) aniba-dimer-A (**3.71**):



Pyrone **3.44** (19.9 mg, 0.026 mmol) was suspended, with stirring, in water and exposed to a sunlamp (15 cm from vial) for two 5 hr periods. Upon noting the loss of starting material in the ^{1}H NMR spectrum, the resulting solid was purified by repeated flash column chromatography (silica gel, 0 to 2% methanol in dichloromethane) to give asymmetric dimer **3.71** as an amorphous colourless solid (9.7 mg, 49 %).

Mp 103-104 ºC; TLC R_f (3% MeOH:CH_2Cl_2) 0.36; IR (smear) ν_{max} 2960, 2906, 2864, 1713, 1626, 1568, 1507, 1487, 1444, 1391, 1240, 1176, 1143, 1109, 992, 961, 828, 695 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.34 (4H, m, H-18,20,18',20'), 7.26 (2H, m, H-13,13'), 7.17 (1H, d, *J* = 7.9 Hz, H-14'), 6.98 (1H, br, H-10'), 6.90 (8H, m, H-10,12,14,17,21, 12',17',21'), 6.85 (1H, m, H-8'), 6.53 (1H, d, *J* = 15.9 Hz, H-7'), 5.89 (1H, d, *J* = 2.1 Hz, H-5), 5.35 (1H, d, *J* = 2.1 Hz, H-3), 5.26 (1H, s, H-3'), 4.28 (1H, t, *J* = 10.3 Hz, H-8), 4.09 (1H, d, *J* = 11.1 Hz, H-7), 3.72 (3H, s, OMe-4), 3.55 (1H, d, *J* = 9.6 Hz, H-5'), 3.34 (3H, s, OMe-4'), 1.32 (9H, s, H-23,23'), 1.32 (9H, s, H-23,23'); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.4 (C-4), 169.7 (C-4'), 164.3, 163.7 (C-2,2'), 158.4 (C-6), 158.0, 157.8 (C-11,11'), 154.3, 154.3 (C-16,16'), 146.5, 146.3 (C-19,19'), 137.6 (C-9,9'), 131.2 (C-8'), 130.0, 129.6 (C-13,13'), 126.6, 126.6 (C-18,20,18',20'), 124.8 (C-7'), 121.8 (C-14[a]), 120.8 (C-14'), 118.6, 118.4, 118.2, 117.9, 117.8 (C-10,12,17,21,12',17',21' [a]), 117.3 (C-10'), 102.6 (C-5), 91.8 (C-3'), 88.8 (C-3), 79.2 (C-6'), 55.9 (OMe-4), 55.5 (OMe-4'), 54.3 (C-7), 45.6 (C-5'), 38.9 (C-8), 34.3 (C-22,22'),

31.5 (C-23,23'); (+)-ESIMS *m/z* 753 $[M+H]^+$, (+)-HRESIMS *m/z* 753.3402 (calcd for $C_{48}H_{49}O_8$ 753.3422).

5. References

1. http://www.who.int/mediacentre/factsheets/fs104/en/index.html (accessed June 25th, **2010**)
2. http://www.who.int/mediacentre/factsheets/fs094/en/index.html (accessed June 25th, **2010**)
3. http://www.who.int/mediacentre/factsheets/fs259/en/ (accessed July 25th, **2010**)
4. http://www.who.int/leishmaniasis/en/index.html (accessed July 25th, **2010**)
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